UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Comparative Environmental Impact Assessment on a Pharmaceutically Interesting Compound

MSc RESEARCH DISSERTATION

Prepared by

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Submitted to

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October 2020

Declaration

I **STEPHANIE GINA AKAKIOS (602385)**, in fulfilment of the requirements of my **MSc Research Dissertation**, declare the following:

This dissertation is my own unaided work, except were due acknowledgement is made to others.

It is being submitted for the Degree of Master of Science in Chemical Engineering to the University of the Witwatersrand, Johannesburg.

It has not been submitted before for any other degree or examination at any other University or academic institute.

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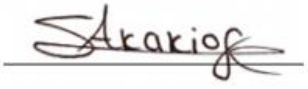
Signature:  **Date:** 30th October 2020

Executive Summary

This dissertation entails an early stage comparative 'greenness' evaluation on three current synthetic pathways to bis-THF alcohol – a key active subunit used in a class of ARV's that shows high efficacy in combating multi-drug resistance of various HIV strains. The three most recent and innovative bis-THF alcohol synthesis routes that were assessed include route A (Hayashi, Aikawa, Shimasaki, Okamoto, Tomioka, Miki, Takeda and Ikemoto, 2016), route B (Sevenich, Liu, Arduengo III, Gupton and Opatz, 2017) and route C (Moore, Stringham, Teager and Yue, 2017). The aim was to find the most eco-efficient bis-THF alcohol synthesis route using green chemistry metrics, principles, guides and web-based tools. The assessment strategy entailed examining each synthetic pathway with specific focus on waste generation (E - factors), process greenness relative to industrial benchmarks (iGAL™), the quantities and nature of solvent used (Solvent Intensity and GSK solvent guide), Green Motion™ and the principles of green chemistry in general. In understanding the challenges that lie in combining assessment methods to effectively compare the greenness between synthetic routes, when faced with green alternatives, the most eco-efficient synthetic pathway was found to be route $B_{\text{entire one-pot}}$ procedure, adhering to most of the green chemistry principles, returning good metric and assessment results whilst further being most economically feasible against its comparators.

Route A reported by Hayashi *et al.*, (2016), involved a stereoselective catalytic cross aldol reaction between polymetric ethyl glyoxylate and a protected aldehyde. Route $A_{\text{entire step-by-step}}$ afforded an overall yield of 54% over 5 synthesis steps, a reaction complexity of 4 and a cumulative E-factor of 14.54.

Route B reported by Sevenich *et al.*, (2017), involved a photocycloaddition between furan and a protected glycol aldehyde, followed by the hydrogenation and lipase catalysed enzymatic resolution. The furan starting materials as well as many other reagents used in the synthetic pathway were reportedly derived from renewable woody biomass. The protected glycol aldehyde was considered an advanced starting material requiring its own synthetic pathway to be assessed. Further, for route B both a step-by-step procedure as well as a one pot procedure were assessed. The route $B_{\text{entire step-by-step}}$ including the advanced starting material synthesis had an overall yield of 24% over 5 synthesis steps, a reaction complexity of 4 and a cumulative E-factor of 126.77. Combining the advanced starting material with the main synthesis as a one-pot procedure afforded an overall yield of 23% over 2 synthesis steps, a reaction complexity of 4 and cumulative E-Factor of 89.69 and more efficient, environmentally and economically more favourable than the step-by-step procedure.

Route C reported by Moore *et al.*, (2017) uses a natural starting material, optically pure monopotassium isocitrate that is obtained from a high-yielding fermentation process, thus eliminating the need for enzymatic resolution and did not require an advanced starting material. The merit of route C was the early positioning of the resolution step. Both a step-by-step and a partial one-pot procedure were

assessed. The overall yield of the former was 50% over 5 synthesis steps, with a reaction complexity of 2 and E-factor of 124.84. For the one-pot procedure the overall yield was 44% over 3 synthesis steps, with a reaction complexity of 2 and E-Factor of 273.37. The one-pot procedure aided in condensing the synthetic pathway but required an increase in solvent quantities thus performing worse environmentally and economically than the step-by-step procedure.

From the iGAL^{TM} methodology, route $A_{entire\ step\text{-}by\text{-}step}$ was found to have 'excellent' mass-efficiency with the lowest E-factor of 14.54, generating 84% and 88% less solvent and water waste than route $B_{one\text{-}pot}$ and $C_{step\text{-}by\text{-}step}$ respectively.

Solvent materials were found to be the largest waste contributors (~ 53%) and the greatest environmental concern in all three synthetic pathways. Thus, solvent utilization offers the greatest potential in reducing the E-factor of bis-THF alcohol synthesis both qualitatively and quantitatively for all pathways. Route $B_{entire\ one\text{-}pot}$ showed that 80% of its total solvent mass were green solvents (EtOAc and MeOH), making it the least impactful, safest and most cost effective synthetic pathway saving up to 51% compared to route A and C procedures. Conversely route C procedures comprised of 95% hazardous and problematic solvents requiring > 500 kg solvents per kg product, resulting in the most costly and wasteful synthetic pathway with health and safety issues.

The Green Motion^{TM} tool predicts route $B_{one\text{-}pot}$ procedure to be the most sustainable owing to its unique combination of green chemistry techniques including one-pot procedure, bio-catalysis, ambient reaction conditions, reasonable amounts of green solvents and renewable raw materials originating from woody bio-mass. Conversely route $C_{one\text{-}pot}$ was predicted to be the least sustainable pathway owing to the variety of energy intensive process operations (i.e., distillation, crystallization, multiple heating and cooling), large amounts of solvent materials used and high waste generation in synthesizing bis-THF alcohol.

From an economic perspective, the total synthesis cost of route $B_{entire\ one\text{-}pot}$ procedure was found to be most economical with route A and C being 1.7 and 4.3 times more expensive respectively.

From a collective and comparative assessment using a radial polygon, it was found that route $B_{one\text{-}pot}$ procedure covered the highest polygon area at 85% thus performing best across multiple greenness assessments. The radial polygon ranked the synthetic pathways from best to worst across multiple greenness assessments as: route $B_{one\text{-}pot}$, route $B_{step\text{-}by\text{-}step}$, route $A_{step\text{-}by\text{-}step}$, route $C_{step\text{-}by\text{-}step}$ and route $C_{one\text{-}pot}$ procedure.

Finally, Route $B_{one\text{-}pot}$ procedure was found to be the most environmentally friendly and economical (eco-efficient) sustainable synthetic pathway to the bis-THF alcohol scoring the overall best amongst all assessment criteria.

Dedication

To Koukla Akakios,

For your unconditional love, support and friendship throughout my studies.

You will forever live in my heart.

And

To Spiro Akakios,

For your exceptional willpower and hard work in completing your Mechanical Engineering degree.

You are my inspiration.

Acknowledgements

I would like to express my sincere thanks and appreciation to the following important people, all of whom made this dissertation possible:

My supervisors, Professor Roger Sheldon and Professor Kevin Harding, for their support, guidance, invaluable knowledge and stimulating comments during the course of this dissertation;

My mother, Loukia, for her continuous love and support and whose academic success has inspired and motivated me in completing this dissertation;

My father, Nick, and brother, Spiro for their unwavering support, assistance and encouragement throughout my study;

My grandmother, Marietta for her unconditional love and belief in me;

My boyfriend, Peter, for his continuous support, encouragement and patience;

My best friend, Argyris, for his consistent encouragement and belief in me;

Professor Till Opatz and colleagues for sparking innovation in the topic of this dissertation;

The National Research Foundation (NRF), for financial support towards this research;

The Mane team for the use of the Green Motion™ tool; and

Frank Roschangar for the inspiration and use of iGAL™ methodology and Green Score cards.

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Abbreviations

General Abbreviations

ACS	American Chemical Society
AE	Atom Economy
AIDS	Acquired Immune Deficiency Syndrome
API	Active Pharmaceutical Ingredient
ART	Antiretroviral Therapy
ARV	Antiretroviral
ASM	Advanced Starting Material
Asp	Aspartate
BASF	Baden Aniline and Soda Factory
Bis-THF	Bis-Tetrahydrofuran
cART	combination Antiretroviral Therapy
$CC5_{antagonist}$	Chemokine receptor antagonist
CE	Carbon Economy
cE-factor	Complete Environmental Impact Factor
CIP	Cahn-Ingold-Prelog
CMR	Carcinogenic, Mutagenic and Reprotoxic
CP	Process Complexity
DRV	Darunavir
ee	Enantiomeric excess
E_{act}	Activation Energy
E-factor	Environmental Impact Factor
FDC	Fixed dose compilation
FLASC	Fast Life Cycle Assessment
FMW	Salt Free Molecular Weight

GAL	Green Aspiration Level
GCIPR	Green Chemistry Institute Pharmaceutical Round Table
GHG	Green House Gas
GHS	Global Harmonized System of Classification and Labelling of Chemicals
GSK	GlaxoSmithKline
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
I	Process Ideality
iGAL	Innovation Green Aspiration Level
INSTI	Integrase inhibitor
KPPI	Key Performance Process Indicators
LCA	Life Cycle Assessment
LR	Limiting reactant
mGAL	Normalization Factor for Innovation Green Aspiration Level
MW	Molecular Weight
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
STR	Single Tablet Regime
PI	Protease inhibitor
PMI	Process Mass Intensity
PoCC	Proof of Clinical Concept
RPG	Relative Process Greenness
SAHPRA	South African Health Products Authority
sE-factor	Simple Environmental Impact Factor
SI	Solvent Intensity
VOC	Volatile organic compound

WHO	World Health Organisation
WWI	Wastewater Intensity

Route A- Advanced starting material synthesis

Bn	Benzyl protecting group
BnCl	Benzyl Chloride
Et_2O	Diethyl Ether
iPrOAc	Isopropyl Acetate
TEMPO	Tetramethyl piperidinyloxyl

Route A- Main synthesis

D2PM	(S)-Diphenylprolinol
MeOH	Methanol
PEG	Polyethylene glycol
PhMe	Toluene
PTSA • H_2O	*p*-Toluenesulfonic acid monohydrate
THF	Tetrahydrofuran
TMOF	Trimethyl orthoformate

Route B- Advanced starting material synthesis

CbzCl	Benzyl chloroformate
Cbz	Carboxybenzyl protecting group
EtOAc	Ethyl Acetate
MeOH	Methanol
Py	Pyridine

Route B- Main synthesis

c-hexane	Cyclohexane
MTBE	Methyl tert-butyl ether
Pd/C	Palladium on activated charcoal
PPL	Procine Pancrease Lipase
$(PrCO)_2O$	Propionic anhydride

Route C- Main synthesis

Ac_2O	Acetic anhydride
$(COCl)_2$	Oxalyl chloride
CPME	Cyclopentyl Methyl Ether
DCM	Dichloromethane
DMF	N,N-Dimethylformamide
EtOAc	Ethyl Acetate
EtOH	Ethanol
Me -THF	Methyl- Tetrahydrofuran
PhMe	Toluene
Py	Pyridine
THF	Tetrahydrofuran

1 Introduction

Pharmaceutical companies are focused on developing active pharmaceutical ingredients (API's) that enhance the longevity and well-being of patients. Since the 1980's, the production of API's has tripled with manufacturing costs exceeding $300 billion in 2007 (Jiménez-González *et al.*, 2012). This led to increased stringency of environmental regulations and legislations. Inevitably, such exponential growth is accompanied with the increased pressure for pharmaceutical companies to adopt sustainable methods in providing patients with affordable medications whilst minimizing their environmental footprint. Implementing green chemistry techniques and principles is thus essential in eliminating the current inefficiencies of operations to help meet the pressures of high consumer demand, increase environmental justice and provide products with a competitive edge.

Antiretroviral (ARV) are chemically synthesized API's used in the treatment of Human Immunodeficiency Virus (HIV). ARV's play a critical role preventing viral transmittance and normalizing the lives of millions of HIV positive patients worldwide. Bis-Tetrahydrofuran (bis-THF) is a common, key building block used in a class of ARV medications known as Protease Inhibitors (PI's), the significance of which lies in their ability to combat multi-drug resistance HIV strains by inhibiting viral replication (Ghosh et al., 2006). Since 1994 extensive research has been conducted on diverse synthetic pathways to the bis-THF moiety. Numerous synthetic pathways reported varied limitations including multiple reaction steps, expensive starting material, hazardous solvents, toxic metal catalysts, limitations to upscaling and/or low stereoselectivity.

Owing to the importance and need for sustainable ARV production and the growing demand to decrease the environmental footprint within the pharmaceutical industry in general, an opportunity lies in finding the most eco-efficient bis-THF alcohol synthetic strategy.

A key area in 'greening' the pharmaceutical industry lies in reduced solvent quantities and innovative API synthetic design. This entails short, practical reactions with early resolution steps that are safe, use low quantities of non-hazardous solvents, biocatalysts, renewable resources and other green technologies including one-pot procedures, xylochemistry and photochemistry at ambient operating conditions.

Early stage assessments of medicinal products and processes (i.e., at conceptual and laboratory scale) are essential before large scale implementation, especially given the short time frame assigned to API synthesis design in pharmaceutical timelines. According to Cespi *et al.*, (2016), the number of publications associated with early stage greenness assessments of pharmaceutical manufacture has increased since 2013, confirming its relevance and interest. Pharmaceutical companies including GlaxoSmithKline (GSK), Pfizer, Novartis, Merck, Boehringer, chemical company Baden Aniline and Soda Factory (BASF), flavour and fragrance company MANE and cosmetics company Chimex have

encouraged and integrated green chemistry principles and techniques into their manufacturing and business practices (Cespi, Cucciniello, Ricciardi, Capacchione, Vassura, Passarini and Proto, 2016).

The term green chemistry was introduced by Paul Anastas and John Warner as a philosophy to design chemical products and processes that reduce or eliminate the use and/or generation of hazardous or harmful substances, using 12 principles. (Cue, 2012). Green chemistry thus aims to reduce the environmental impact of chemicals manufacture by promoting the use of safe, non-toxic chemical materials encouraging the use of renewable raw materials, energy efficient techniques and waste prevention rather than waste remediation methods. It is in alignment with the triple bottom line of sustainability in meeting societal (people), ecological (planet) and economic (profit) needs by manufacturing robust, high quality products at low costs meeting environmental regulations (Koenig and Dillon, 2017and Sheldon, 2017b).

The green chemistry movement provided momentum towards the development of a sustainable chemical industry with the 12 principles being the reference for every publication and initiative for greener production (Phan, Gallardo and Mane, 2015). These principles provide efficient guidelines in developing less environmentally impactful processes with 'life cycle thinking' at the onset of the synthesis design (Anastas and Lankey, 2000). For instance, an ideal pharmaceutical synthetic design would entail the use of inexpensive chemical materials from renewable resources, in a single reaction step that is practical, safe and environmentally benign with zero waste production (Environmental Impact (E)-factor = 0). In reality however, pharmaceutical synthesis often requires multiple synthesis steps, advanced starting materials and undesired solvents that on average may exceed 100 kg's for 1 kg API (Roschangar, Sheldon and Senanayake, 2015). This draws heavily on natural resources and generates large amounts of waste with adverse effects on the environment. Additionally, there is an absence in acknowledging significant cost implications associated with waste resources (mass and energy) which corresponds with poor reaction mass efficiencies of synthetic pathways (Constable, Curzons and Cunningham, 2002).

As stated by (Roschangar, Sheldon and Senanayake, 2015) "pharmaceutical companies do not always reflect the best possible molecular assembly strategies" in developing a desired pharmaceutical compound. This outcome has the consequence of higher environmnetal and economic costs than might be otherwise achieved using alternative strategies. Thus, performing early stage quantitative assessments on alternative synthesis routes is of great significance in providing patients with more affordable medication whilst minimizing environmental impacts. The twelve principles of chemistry coupled with green chemistry metrics, solvent guides, metrics and web-based tools offer chemists and chemical engineers' guidance in identifying the greenest synthetic pathway when faced with alternatives and trade-offs.

The successful implementation of green chemistry lies in translating them into mathematical equations (Andraos and Sayed, 2007). Green chemistry metrics and web-based tools are good initial quantitative measures of process greenness. As the proverb says, 'you can't manage what you don't measure' thus implying that metrics and tools are of utmost importance in conveying greenness performance. According to Sheldon (2012), "none of the alternative green mass metrics offer great advantage over the E-factor for describing how wasteful a process is." This is particularly true when considering that waste elimination has always been the major driving force behind the green chemistry movement.

As Protti, Fagnonia and Albini, (2018) stated: 'A commitment to health is incomplete without the commitment to a healthy environment'. Green chemistry and engineering play a vital role in influencing more sophisticated pharmaceutical research and manufacturing methods. It supports medical innovation, human and environmental safety and aids the production of affordable medicines that will help build a resilient health care industry for future generations.

1.1 Problem Statement

To evaluate and compare the environmental and economic performance of current, bis-THF alcohol synthesis routes using green chemistry metrics, principles and green web-based tool design (Green Motion^{TM} and green scorecards). The challenge lies in combining assessment methods to effectively compare the greenness between synthetic routes (when faced with green alternatives and trade-offs) to identify the most eco-efficient bis-THF alcohol synthesis route. The most environmentally friendly synthetic pathway should adhere to as many of the green chemistry principles as possible, return good metrics, assessment results and be economically feasible.

1.2 Research Objectives

The research objectives of the project are:

a. Select three suitable synthetic routes to bis-THF alcohol for analysis;
b. Assess the synthetic pathways using green chemistry metrics and tools;
c. Evaluate and compare the environmental impact of the alternative bis-THF alcohol synthesis routes;
d. Evaluate and compare the economics of the alternative bis-THF alcohol synthesis routes; and
e. Identify the "greenest" (eco-efficient[1]) bis-THF synthesis route.

[1] Eco-efficiency defined as maximized productivity with minimized environmental impact and expense of the chemical procedure (Bengtsson, 2004).

1.3 Research Questions:

The research questions for the project are as follows:

a. Are solvents the greatest mass contributors in the bis-THF alcohol synthetic pathways?
b. What qualitative (solvent analysis, Green Motion™ and 12 principles of green chemistry) and quantitative (E-factor, green metrics and iGAL™) are required in assessing process greenness?
c. Which bis-THF alcohol synthesis route is the most environmentally friendly and economical (eco-efficient)?

Since there is no single green chemistry metric or tool that incorporates the multiple facets of process greenness and sustainability at the manufacturing stage of API's; a selected combination of green chemistry metrics, tools and guides were used to provide a well-rounded comparative environmental impact assessment in synthesizing bis-THF alcohol.

2 Literature Review

2.1 HIV and Bis-THF Alcohol

In 1981, the first case of Acquired Immunodeficiency Syndrome (AIDS) was reported in the United States. Since the onset of the epidemic, 76.10 million people have been infected globally with sub-Saharan Africa accounting for two-thirds of the global infection (Mudgal, Birudukota and Doke, 2018). According to the World Health Organization (WHO), 36.70 million people worldwide were living with HIV in 2016 with approximately 21.70 million people receiving effective antiretroviral therapy (ART) in 2017, an exponential increase from 2.10 million individuals receiving treatment in 2005 (World Health Organization, 2019). ART's have the primary objective of keeping HIV patients alive and the secondary objective to prevent viral transmittance. According to the WHO (2016), between 2001 and 2011 antiretrovirals (ARV's) have reduced the global HIV transmittance by 20%. Despite this, 60% of HIV positive individuals living in Southern and Eastern Africa still do not have access to ARV's (Child, 2019). This increases the demand for low-income countries to have sustainable methods of manufacturing ARV's making them readily available.

Over the past 30 years, scientists have achieved significant advances in the treatment of HIV and AIDS. From no available therapies at the onset of the virus, to the development of active ingredients that have controlled the replication of the virus, thus rehabilitating the lives of millions. In recent years, there has been a consistent trend in expanding the range and use of ARV's in less developed countries where the infection remains prominent. This trend with the Joint United Nations Program ambitious 90-90-90 HIV target strategy of 2020 (i.e., for 90% of individuals living with HIV to know their status, 90% of infected individuals to receive sustained ARV treatment and for 90% of those infected to have viral suppression) is to end the HIV epidemic by 2030 (UNAIDS, 2019). To achieve these targets, estimated budgets of $26.20 billion and $22.30 billion US dollars will be required by 2020 and 2030 respectively (UNAIDS, 2016).

AIDS is a chronic disease caused by the HIV retrovirus when left untreated (Lange and Ananworanich, 2014). The virus stores its genetic material in the ribonucleic acid (RNA) which is then converted into double stranded deoxyribonucleic acid (ds DNA) and then integrated into host DNA. Cellular machinery then produces the new proteins and new viral genomic RNA copies (Seitz, 2016). The HIV infection slowly destroys a human's immune system by destroying white blood cells, known as CD4-cells, increasing the risk for other infections and diseases. Typically, a healthy CD4-cell concentration ranges between 500 - 1600/mm^3 of blood, whereas an HIV patient is diagnosed with AIDS when the CD4-cell count falls below 200/mm^3 (AIDS InfoNet, 2014). ARV's are medications used to control the HIV virus by maintaining a healthy CD-4 cell count. There are two strains of the virus namely HIV-1 and HIV-2. The HIV-1 strain is more prevalent worldwide with ARV development and production focused on combating this strain (Appendix A.1).

Currently ART is the standard treatment of HIV which comprises a combination of ARV drugs from different pharmacological classes. The different drug classes are designed to suppress viral transmittance at distinct stages of the viral reproductive cycle. This slows down the progression of the disease and boosts the immune system. Treatment using customized combination of different drug classes is commonly referred to as Highly Active Antiretroviral Therapy (HAART) or combination antiretroviral therapy (cART). These combination strategies commenced in 1995 and continue to be effective in increasing the life expectancy of HIV patients by two decades or more (Voshavar, 2019). Common ARV drug classes used in HAART include: protease inhibitors (PI's), integrase inhibitors (INSTI's), nucleoside reverse transcriptase inhibitors (NRTI's), non-nucleoside reverse transcriptase inhibitors (NNRTI's), fusion inhibitors (FI's) and chemokine receptor antagonists (CCR5) (Rathbun, 2019). Amongst these various drug classes, PI's are widely used in treating HIV (Voshavar, 2019).

There is no cure for HIV, however HAART, a current therapy using the combination of at least three ARV drugs, effectively manages the progression and transmission of the HIV infection and has indeed improved the health and quality of life of those infected. More specifically, HAART with combination of PI's and reverse transcriptase inhibitors continues to be an effective treatment in controlling the HIV infection (United Nations, 2004 cited in Ghosh et al., 2006). Despite these impressive successes, the development of multi-drug resistant HIV strains in patients remain the most serious and challenging problem in current medicine discovery (Wainberg & Friedland, 1998 cited in Ghosh et al., 2006). Owing to this challenge, much research and development has been devoted to both existing and new classes of HIV PI's. These inhibitors are designed to combat drug resistance by interacting with the backbone of the HIV protease active site and currently hold 60% of the average market share for adult ARV drugs (Ghosh et al., 2006 and World Health Organization, 2016a).

Figure 1 illustrates a structural representation of a group of new generation HIV PI's including brecanavir, GS-8374, SPI-256 as well as Food and Drug Administration (FDA) approved duranavir (DRV). These second generation compounds were designed to overcome problems posed by the first generation compounds and were reported to show high efficacy in combating multi-drug resistance (Sevenich, Liu, Arduengo III, Gupton and Opatz, 2017). More specifically, DRV showed robust interactions with the protease enzyme on various HIV strains, including strains from treatment experienced HIV patients (Ghosh, Sridhar, Leshchenko, Hussain, Li, Kovalevsky, Walters, Wedekind, Grum-Tokars, Das, Koh, Maeda, Gatanaga, Weber and Mitsuya, 2006).

More importantly, Figure 1 shows a common key side chain (boxed in dark green) that is found in a class of commercial and experimental HIV drugs known as PI's. The common key side chain has an International Union of Pure and Applied Chemistry (IUPAC) name of (3R,3aS,6aR)-Hexahydrofuro [2,3-*b*] furan-3-ol, but is widely known and abbreviated as bis-THF moiety.

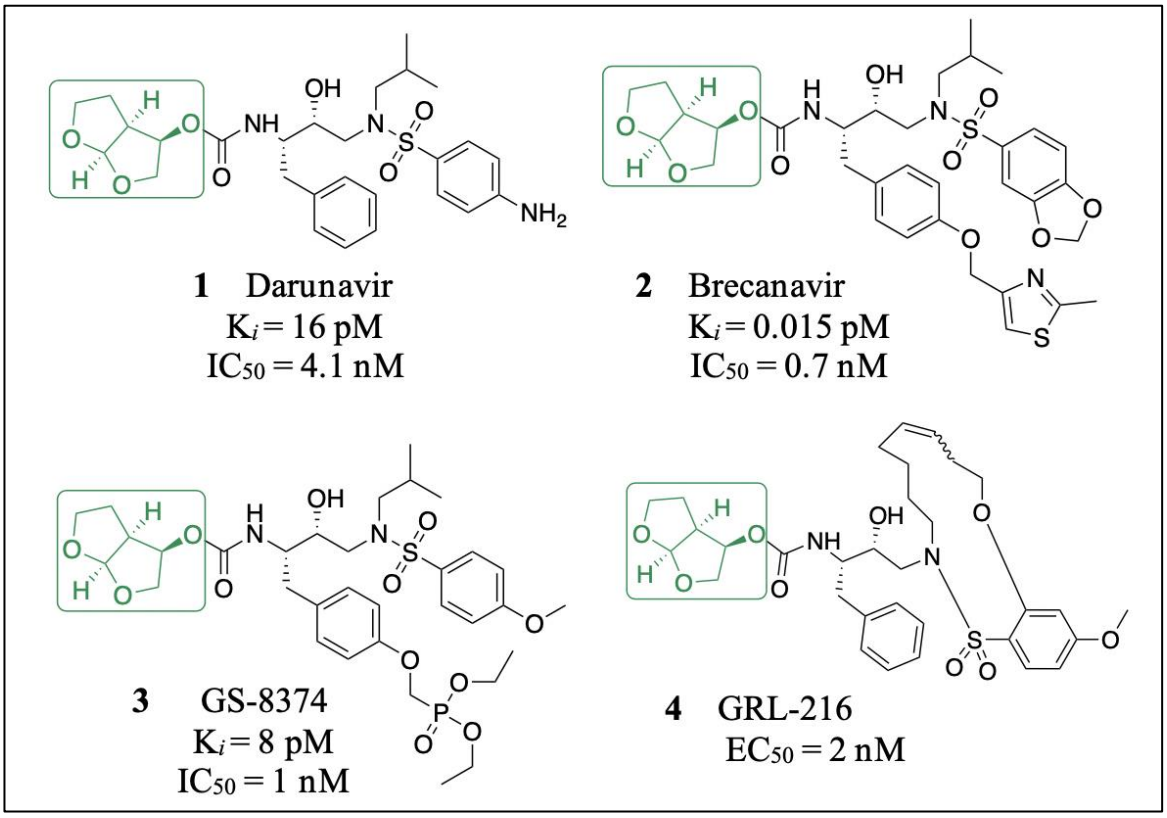


Figure 1: Structural representation of new generation HIV PI's 1-4 highlighting bis-THF moiety (dark green) (Sevenich et al., 2017).

The bis-THF moiety is referred to as the "privileged ligand" due to the compound being well documented in combating drug resistance (Ghosh, Li, & Perali, 2006 cited in Sevenich et al., 2017). From Figure 1, DRV is currently the only FDA approved HIV PI that contains the key bis-THF subunit with other drug compounds Brecanavir, GS-8374 and GRL-216 still undergoing clinical trials.

Further, Figure 2 illustrates a three dimensional X-ray crystal image[2] of the interaction between the bis-THF moiety and the backbone of the HIV-1 protease enzyme. The effective hydrogen bond formation between the bis-THF moiety (purple) and the enzyme active site (amino acid nucleophiles Aspartate (Asp) 29 NH and Asp 30 NH (green)) is considered a key target for the inhibition of viral replication. The success is critically attributed to the bis-THF moiety's conformational rigidity, ring stereochemistry and positioning of oxygen atoms (Ghosh et al., 1996; Ghosh et al., 2006). The numerical values illustrated in Figure 2 are the hydrogen bond lengths in Angstroms ($\dot{A}$).

[2] X-ray crystallography is a technique used to show the binding between the enzyme active site and the drug substance. It reveals the structure, function and chemical interactions of chemical and biological molecules (Scapin G, 2006).

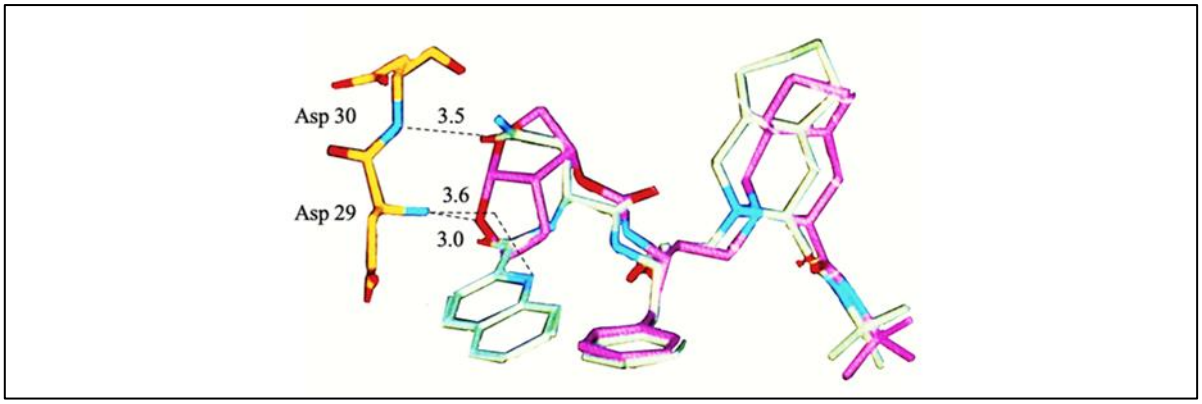


Figure 2: X-ray crystal structure of HIV enzyme - protease inhibitor complex (Ghosh et al., 1994).

According to WHO (2016), DRV was the favoured PI active ingredient in second and third line ART. It has proven clinical efficacy in both treatment of naïve and experienced HIV infected patients (Sued, Figueroa, Gun, Belloso, Cecchini, Lopardo, Pryluka, Rolon, Fink, Lloret and Cahn, 2017). This is owed to its advantage of lower toxicity levels and higher resistance profiles compared to other PI active ingredients, making it notably used in developed countries (Moore, Stringham, Teager and Yue, 2017). The demerit of the bis-THF alcohol however is its costly manufacture that may contribute to more than half of the complete ARV medication cost (Moore, Stringham, Teager and Yue, 2017). Greater detail pertaining to the chemical structure of PI's and bis-THF moiety and their interactions with the HIV protease active site is described in Appendix A.2.

Further, DRV is an oral non-peptidic HIV-1 PI that is sold under the brand name Prezista™. It forms part of HAART when co-administered with ARV's cobicistat or ritonavir (McKeage, Perry and Keam, 2009). In July 2018, Johnson & Johnson announced the FDA approval of Symtuza™ - a DRV based single-tablet-regime (STR) for the treatment of HIV-1. Symtuza™ has four active ARV ingredients in one tablet. It is thus a fixed dose combination (FDC) tablet with a ratio of 800 mg DRV, 150 mg cobicistat, 200 mg emtricitabine and 10mg tenofovir alafenamide (Johnson and Johnson, 2018). The tablet is administered once daily and designed to prevent multiple drug misuse thereby reducing drug resistance. According to AIDS research centre in North Carolina, phase three clinical trials of Symtuza™ concluded a 95% virologic suppression for ARV treatment naïve patients, demonstrating the pharmacological efficacy of the STR technology (Johnson and Johnson, 2018). The STR formula thus supports the implementation of sustained ARV treatment for HIV infected individuals. For an adult HIV patient that has no drug resistance, DRV is prescribed as an 800 mg tablet taken once daily (Janssen Pharmaceuticals, 2016). A kilogram of bis-THF alcohol thus provides 2 patients with a yearly supply of DRV. The DRV molecule was estimated to last 97.50 hours in the human body (Appendix B.2 and B.3). The new STR technology has promising potential to lower the ARV demand for HIV positive patients worldwide.

Table 1: Aggregate of Darunavir (DRV) tablets required by patients per year from 2016 – 2019 (World Health Organization, 2016a)

Darunavir concentration (mg)	Number of patients per year			
	2016	**2017**	**2018**	**2019**
150	240	653	1241	1926
300	130	173	182	191
600	466	655	877	1086

Table 1, illustrates an increase in the number of HIV positive patients requiring DRV medication over the past few years. The aggregates were declared by the Supply Chain Management System and the Government of South Africa (World Health Organization, 2016a) and shows DRV as a notably used ARV in developed countries. The increased need for DRV medication thus demands the need for sustainable DRV manufacturing practices. The wider use of DRV however remains repressed owing to its costly manufacture especially in less resourced locations. More specifically, a cost analysis performed on numerous synthetic pathways to DRV showed that the bis-THF moiety contributed to as much as half the cost in synthesizing the DRV active ingredient (Moore, Stringham, Teager and Yue, 2017). This limits the interest of generic manufacturers and lowers production volumes (Appendix A.3). Consequently, the implementation of economies of scale to reduce product prices and encourage wider use is hindered. Thus, finding a cost-effective and environmentally favourable bis-THF alcohol synthesis is an asset in its operation.

The importance of bis-THF alcohol in HIV PI treatment has led to the development of numerous routes for its synthesis. Medicinal target molecules are generally of high molecular complexity requiring multiple chemical steps and thus longer synthetic procedures. A history of bis-THF alcohol synthetic pathways is illustrated in Appendix C.1.

Since 1994, organic and medicinal chemists have published numerous synthetic pathways to the bis-THF alcohol, each with the incentive to be in some aspect more advantageous than those previously published (i.e., environmentally favourable, decreased synthetic and purification steps, increased yield, lower cost, increased stereoselectivity etc.). These synthetic alternatives are continually being devised owing to the compound's importance in providing a therapeutic effect to HIV positive patients and its costly synthetic nature (Moore, Stringham, Teager and Yue, 2017). Some of the publications on the alcohols synthetic pathway include: Ghosh *et al.*, 1994, 2011; Ghosh and Chen, 1995; Uchiyama *et al.*, 2001; Quaedflieg *et al.*, 2005; Yu *et al.*, 2007; Black *et al.*, 2008; Canoy *et al.*, 2008; Kulkarni *et al.*, 2010; Hayashi *et al.*, 2016; Moore *et al.*, 2017; Sevenich *et al.*, 2017.

A brief review of previously reported bis-THF alcohol synthetic studies are discussed below, the schematic representations and route numbers of which are illustrated in Appendix C.1. Each subsequent innovation demonstrates increased awareness towards human and environmental safety and well-being and in some instances cost reduction. The original reference of each synthesis route (illustrated in Appendix C.1) includes the experimental procedure providing the amounts and types of chemical materials (reagents, solvents, water, catalysts etc.) used.

Appendix C.1 shows that the earlier reported bis-THF alcohol synthetic pathways focused on API purity and yield with more recent synthetic pathways focusing on environmental awareness, cost reduction and acceptable enantiomeric purity. Owing to the most current synthetic pathways route A (Hayashi, Aikawa, Shimasaki, Okamoto, Tomioka, Miki, Takeda and Ikemoto, 2016)), route B (Sevenich, Liu, Arduengo III, Gupton and Opatz, 2017)) and 12 route C (Moore, Stringham, Teager and Yue, 2017)) were selected as the three bis-THF alcohol chemical schemes to be investigated in this study.

Many of the earlier reported synthesis routes (D, E and H as in Appendix C.1) exhibit the demerits of using expensive starting materials, and large quantities of hazardous solvents resulting in low yields and purities. These earlier reported bis-THF alcohol synthetic pathways thus focused on API purity and yield sometimes using metal catalysts which often pose problems on scale-up. For example, the use of homogeneous metal catalysts (catalysts in the same phase as the reactants and products) can result in heavy-metal contamination of the final product and heterogenous metal catalysts (catalysts as solids in different phases to reactants and products) often required impractical reaction temperatures ($> 400℃$), low selectivity and unwanted side reactions (Ciriminna, Pagliaro and Luque, 2019). Hence, metal catalysts often have low acceptance in pharmaceutical operations (Belecki and Gupton, 2015) and require additional operational steps to attain acceptable purity of pharmaceutical intermediates and products (typically >99%). More specifically synthesis route E and L were not suitable for large scale due to low operating temperatures and ozone oxidation (Ghosh & Chen, 1995).

A diastereo- and enantio-selective synthesis of the bis-THF alcohol using an Evans Mukaiyama aldol reaction was reported (route J). This synthesis route was efficient but encountered the problem of incorporating heavy metal catalysts with low product purity (Black, Davis, Doan, Lovelace, Millar, Toczko and Xie, 2008). In contrast, recently reported synthesis route 10, entailed environmentally benign organocatalysis in an aldol reaction (asymmetric synthesis), resulting in moderate diastereo - and enantio-selectivity but proved a highly practical synthesis route to the bis-THF alcohol through chiral catalysis in high *ee* purity of $>99\%$ (Hayashi, Aikawa, Shimasaki, Okamoto, Tomioka, Miki, Takeda and Ikemoto, 2016).

Additionally, synthesis route B demonstrated a clean chemical synthesis by employing photochemical reactions in place of elevated temperatures, pressures or aggressive chemical reagents for activating

starting materials. This reaction was a photocycloaddition which is environmentally favourable due to its mild operating conditions, zero formation of spent reagents and residue which consequently made purification processes simpler (Protti, Dondi, Fagnonia and Albini, 2009). Synthesis route B further incorporates the use of wood based starting materials in a one-pot procedure (i.e., one reactor) along with catalytic hydrogenation and enzymatic optical resolution. This concise and effective synthesis route was reported to afford the bis-THF alcohol in high purity of *ee* 99% (Sevenich, Liu, Arduengo III, Gupton and Opatz, 2017).

The most recent synthetic pathways focus on synthesizing a racemic mixture of the bis-THF alcohol followed by an enzymatic resolution. The main issue with this type of synthetic approach is that half of the product is discarded owing to it having the wrong spatial orientation (geometry), thus resulting in a lower yield. Route C was designed with the aim of achieving a "practical stereospecific synthesis" of the bis-THF alcohol without using enzymatic optical resolution and inexpensive reagents to maximize cost effectiveness (Moore, Stringham, Teager and Yue, 2017). The synthesis route entails the use of optically pure isocitric acid obtained from a fermentation process as one of the starting materials thus eliminating the need for kinetic resolution at a later synthesis stage and producing optically pure bis-THF alcohol directly with an *ee* of 96.80% (Moore, Stringham, Teager and Yue, 2017). An extension of the synthesis route from the bis-THF alcohol to the FDA approved DRV was also reported as schematically represented.

The three selected bis-THF alcohol pathways A, B and C made use of innovative green chemistry techniques including: photochemistry (using sunlight as a renewable resource to initiate chemical reactions), one-pot procedures (multiple reactions carried out simultaneously in a single reaction vessel) the use of renewable materials based on xylochemistry (materials derived from woody biomass), bio-catalysis (use of isolated enzymes or living cells to perform chemical transformation), industrial symbiosis (using the waste stream created by one process as the starting material for another process) and varied positioning of the kinetic resolution step, to name a few. These greener technologies claimed to generate greener products and processes resulting in fewer synthesis steps, milder operating conditions and lower waste carry through than previously reported bis-THF alcohol synthetic procedures. The details of these innovative green chemistry techniques are described in greater detail in Appendix C.2.

The reported experimental information of each synthesis provides the basis for conducting green assessments (Ranke, Bahadir, Eissen and König, 2008). Performing these assessments at an early stage of a process allows scientists and engineers to identify environmentally unfriendly aspects of the process and find greener solutions. Being proactive in finding an alternative greener solution implements smarter process design in minimizing environmental impacts and costs at later development stages.

Hence, assessing environmental and economic aspects at early bis-THF alcohol synthetic stages creates a strategic pathway to building a sustainable process.

Another area of low acceptance in pharmaceutical production is the incorrect chirality of the drug molecule. Chirality refers to molecular structures that are non-superimposable on their mirror image that usually contain one or more chiral centres (Clayden, Greeves and Warren, 2012). The mirror images of the chiral molecules are known as enantiomers. The optical purity of a chiral molecule is described by its enantiomeric excess expressed as a percentage (*ee*%) as previously mentioned. Enantiomers or stereoisomers are molecules that have identical chemical and physical properties but differ in 3D spatial orientation (stereochemistry). In other words, enantiomers are two molecules that consist of the same groups of atoms that are connected differently around the chiral centre. Diastereomers are stereoisomers that are not mirror images and may be chiral or achiral. Diastereomers have different physical properties and are thus considered as two completely different compounds that may be easily separated using chromatography (Clayden, Greeves and Warren, 2012). To differentiate between the various spatial orientations, chemists use the Cahn-Ingold-Prelog (CIP) priority rules to describe chiral centres as *(S)* or *(R)* enantiomer nomenclature. The bis-THF alcohol contains three contiguous chiral centres and, hence, eight (2^3) stereoisomers (Appendix C.3). The spatial orientation of the bis-THF alcohol that exhibits therapeutic effectiveness in enantiomeric notation is the (3R,3aS,6aR) - Hexahydrofuro [2,3-*b*] furan-3-ol geometry (graphically illustrated in Appendix C.3).

Synthetic pathways A, B, and C to the bis-THF alcohol afford product with *ee* of >99%, 99% and 96.80% respectively (Hayashi et al. (2016); Sevenich et al., (2017) and Moore *et al.*, (2017)). Synthetic pathways that yield higher percentage purity of the desired API enantiomer are preferred as purification steps reduce the reaction yield of the synthesis.

Since enzyme receptors in the human body therapeutically respond to single (pure) enantiomers, it is essential that synthetic procedures focus on producing enantiomerically pure medicinal compounds. The chiral pool strategy is a commonly used method to synthesize single enantiomers, however it requires enantiomerically pure starting materials which can be costly if not naturally occurring (route C has a naturally occurring enantiomerically pure starting material, potassium isocitrate thus eliminating the need for a resolution step at the onset of the synthesis, which eliminates the waste resulting from the ballast of the wrong enantiomer in subsequent steps and yields an optically pure product directly without the need for enzymatic resolution). Chemo-enzymatic synthetic procedures are also commonly used whereby selective enzymes aid in producing the single enantiomer (Patel, 2007). Other synthetic procedures with racemic mixtures requiring an additional resolution step to separate the mixture into its single enantiomers are route A and B (Clayden, Greeves and Warren, 2012). Separation of enantiomers using resolution inherently produces 50% of the product material as waste since the

undesired enantiomer is discarded if it cannot be re-racemized and recycled. This separation method may be difficult as enantiomers possess similar physical properties (i.e., melting point, boiling point, polarity and so on). Chromatographic methods are also used as effective separation techniques however these techniques require large amounts of packing material and solvent use which is expensive and environmentally unattractive and thus avoided at large scale operations. The choice of strategy in achieving the correct chirality thus has an effect on the green chemistry, e.g., the use of racemates increases waste production during resolution steps in contrast to stereoselective reactions of prochiral substrates that yield single enantiomers. The golden rule with chirotechnology is to plan the resolution step as early as possible in the synthesis as reactions with racemates require double the amount of reagents, solvents etc (Sheldon, 1993). Furthermore, molecular stability is a key characteristic that should be monitored throughout the synthetic procedure. Sometimes, protection groups need to be added to achieve stability and removed at a later stage of the synthesis (as in route A and B). The deprotection step then becomes the key focus in the synthesis design (Jiménez-González and Constable, 2011).

2.2 Greenness within the Pharmaceutical Industry

Over recent years there has been increased interest and awareness in waste reduction, pollution prevention and contribution to climate change within chemical industries. More specifically the pharmaceutical industry has less attentiveness in its environmental practices which has created an illusion of the pharmaceutical industry being relatively green. Belkhir and Elmeligi (2018) attributed this to the minimal historical trends reported on pharmaceutical emissions as well as to the creation of in-house environmental policies within each pharmaceutical company. This results in inconsistent environmental practices, sustainability measures and greenwashing[3]. Furthermore, varied methods of reporting waste production and emissions does not allow for fair comparisons and claims to be made amongst pharmaceutical companies on their medicinal products. Consequently there exists a large variability in emission intensity[4] with the highest pharmaceutical emitter (Eli Lilly at 77.30 Mt-CO_2e/\$M) emitting more than 5 times the lowest emitter (Roche at 14.00 Mt-CO_2e/\$M) in generating equal revenue from similar medicinal product sales (Belkhir and Elmeligi, 2018). Thus the pharmaceutical industry should allow for some transparency and issue "sustainability statements" that follow a standardized and comparable format (Belkhir and Elmeligi, 2018).

[3] Greenwashing is the term used to describe the unsubstantiated or misleading claim of a company's beneficial environmental performance in their products, services or practices (Jiménez-González and Constable, 2011).

[4] Emissions intensity measures the direct and indirect greenhouse gas (GHG) emissions in metric tons of CO_2 equivalent per total revenue in millions of US dollars (Mt CO_2-e/\$M) (Belkhir and Elmeligi, 2018).

As illustrated in Figure 3 the global pharmaceutical sector needs to reduce its emissions intensity by 28% by 2025 to meet the Paris Agreement[5] mitigation targets. As a result, Environment Canada has implemented a carbon pricing strategy to reduce their pharmaceutical sectors carbon footprint of 218 million Mt CO_2-e by 2025 (Belkhir and Elmeligi, 2018).

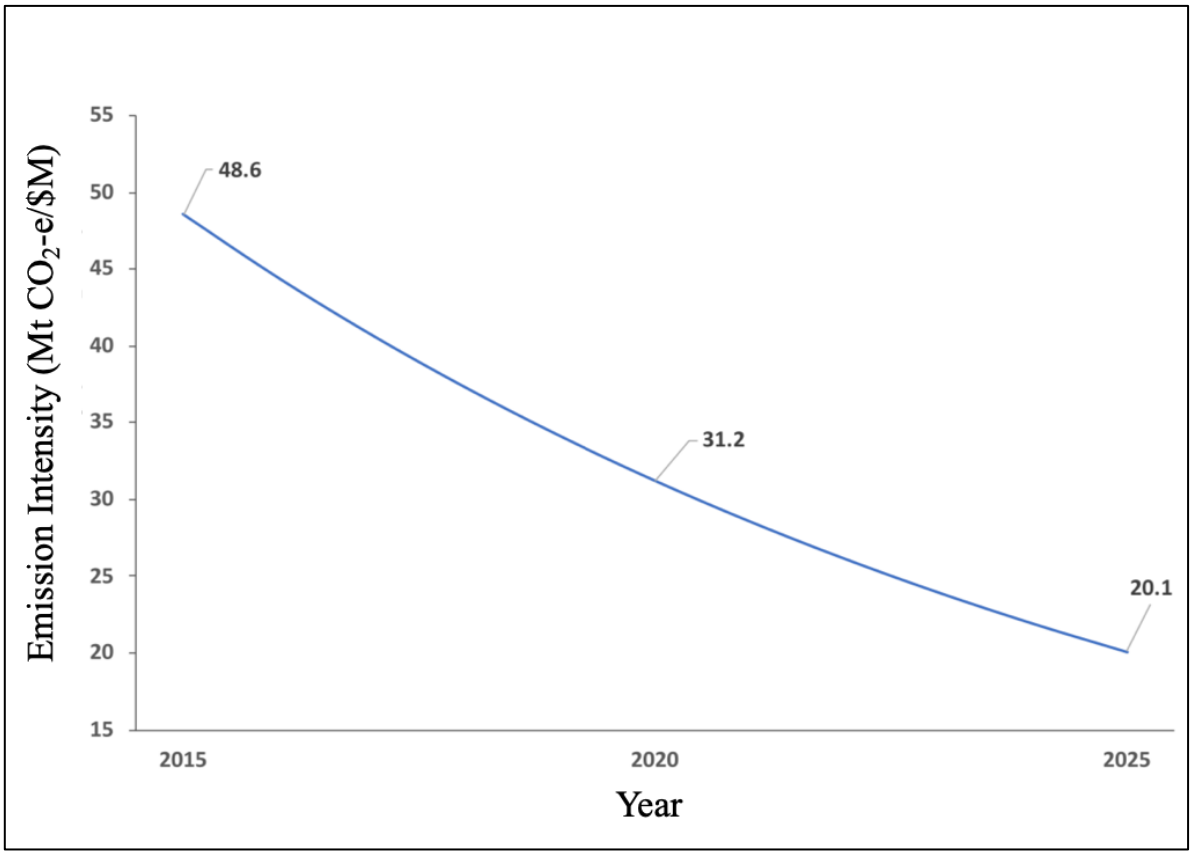


Figure 3: Projection of emission intensity reduction of global pharmaceutical sector required to meet Paris Agreement targets (Belkhir and Elmeligi, 2018).

Drug manufacturing has received much attention in terms of its environmental impact. For example the daily waste produced in manufacturing a broad spectrum of antibiotics is ~ 44 kgs per kg of product (Larsson, 2014).

Figure 4 represents the emission intensity of 15 pharmaceutical companies as well as the emission targets for years 2015, 2020 and 2025. The figure shows that companies such Roche and Johnson & Johnson have significantly reduced their emissions already meeting or exceeding 2025 targets whilst maintaining revenue growth. In contrast Larsson (2014) reported that pharmaceutical companies do not

[5] Paris Agreement is a United Nations agreement signed by the worlds countries in 2016 to fight climate change. It is focused on reducing greenhouse gas emissions, mitigation, adaptation and finance (United Nations Treaty Collection, 2016).

often document their waste generation or discharges to the environment despite having reached extreme levels.

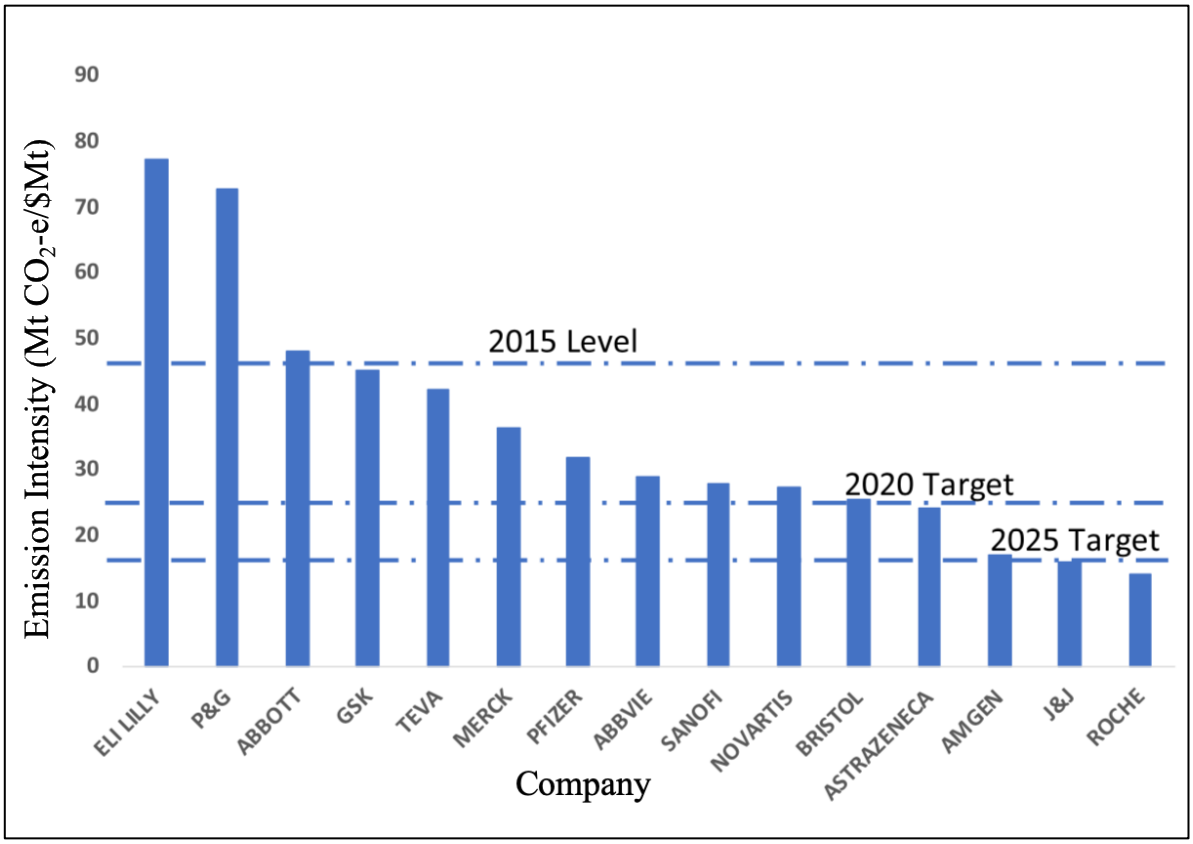


Figure 4: Emission intensity of 15 major pharmaceutical companies in 2015 along with the projected target emission intensities for 2020 and 2025 as per Paris Agreement (Belkhir and Elmeligi, 2018).

Sheldon, (1992) is well-known for having tabulated benchmarked values of waste generated across a number of chemical industries as illustrated in Table 2. The waste generation was measured using his environmental impact (E) - factor metric which calculates the kilograms of waste generated per kilogram product (discussed in detail in Green Metrics, Section 2.4). There exists a wide range of chemical processes producing a wide variety of products ranging from bulk chemicals to fine chemicals and pharmaceuticals. The chemicals are assigned specific categories based on their production volume, functionality, and cost (Tufvesson *et al.*, 2013). Fine chemicals and pharmaceuticals have specialized applications and are generally regarded as high-value chemicals with small to moderate production quantities. Conversely, bulk chemicals are generally low-value chemicals produced in large quantities. Due to these opposing processes, key process parameters including process intensity, degree of optimization, environmental, economic and social impacts, greatly differ (Tufvesson *et al.*, 2013).

As demonstrated in Table 2 the pharmaceutical industry has a substantially elevated waste burden per unit product compared to other chemical segments. The high environmental impact of 25 - > 100 kgs

per kg product for pharmaceutical compounds may be attributed to their high molecular weight and complexity, need for advanced starting materials (ASM's), large quantities of solvents used and /or multiple synthesis steps to assemble the desired API (Roschangar, Sheldon and Senanayake, 2015). Conversely other chemical segments target much simpler molecules thus requiring fewer synthesis steps and consequently less waste generation with more favourable environmental performance.

Table 2: E-factors, waste and process complexities for a range of chemical industries (as adapted from Roschangar, Sheldon and Senanayake, 2015).

Industry segment **(examples)**	**Yearly production** **(tons)**	**E-factor** **(kg waste/ kg product)**	**No. synthetic steps**
Pharmaceuticals (antibiotics, drugs, vaccines)	10 - 1000	25 - > 100	6 +
Fine chemicals (coatings, electronic parts)	100 - 10 000	5 - > 50	3 - 4
Bulk chemicals (plastics, polymers)	10 000 - 1000 000	< 1 - 5	1 - 2
Petrochemical (solvents, detergents)	1 000 000 - 100 000 000	~ 0.1	Separations

Confirming and expanding on Sheldon's, (2015) values for pharmaceutical waste, Koenig and Dillon, (2017) reported that ≥150 kilograms of waste is generated per kilogram of API produced (with purity >99.30% *ee*) at an early development stage. Further, according to the American Chemical Society (ACS) Green Chemistry Institute Pharmaceutical Roundtable (GCIPR), the average benchmarked E-Factor for API's is 130.

Branded and generic pharmaceutical industries produce ≥ 100 million kilograms of API and more than 15 billion kilograms of co-produced waste per year (Cue, 2012). This could lead to pharmaceutical industries being faced with a hefty annual waste disposal cost of approximately $30 billion (Koenig, Mergelsberg and Roschangar, 2017). This provides an incentive for the pharmaceutical industry to implement green chemistry principles and techniques to reduce the waste burden and thereby treat patients with more affordable medication whilst minimizing the environmental footprint.

Moreover, the pharmaceutical industry measures material efficiencies using the E-factor and Process Mass Intensity (PMI) metric. The ACS GCIPR determined the contribution of material impacts in API production from 10 pharmaceutical member companies. It was found that solvents contributed to over half (56%) of the process waste followed by process water (32%) and reactants forming the balance (ACS Green Chemistry Institute, 2014). In other words, in producing 1kg of API using 100 kg of materials >85% of these materials are solvents and water, making these materials the largest waste

source in API production. This stresses the need for reducing the amount of hazardous solvents used and researching the influence of greener alternatives. Despite this, Roschangar, Sheldon and Senanayake, (2015) stated that the impact of the small percentage waste contribution of reactants (7%) and raw materials (<5%) is greater than what is reflected as these compounds may not be easily re-used or recycled.

In 2005 the ACS GCIPR was created for pharmaceutical industries worldwide to connect and share their green techniques with the aim of creating a greener and more sustainable pharmaceutical sector worldwide (Dunn, Wells and Willams, 2010). Over the years the GCIPR has made numerous achievements in academia and industry and continues to provide training and sharing of practical tools to further improve the greening of the pharmaceutical industry (Koenig and Dillon, 2017). The GCIPR has proposed four factors that form a strategic pathway in implementing a greener global pharmaceutical industry, namely 1) influence research agenda 2) create tools for innovation 3) promote education and training and 4) enable global collaboration.

Over the past few years, pharmaceutical industries have implemented green strategies and technologies at the manufacturing stage of medicinal products. This is in alignment with minimizing the industries high waste generation, reducing material and waste management costs, aiming to reach sustainable process design. In addition to the large quantities of solvents used (~ 56%) at the manufacturing stage, extensive amounts are also used during the cleaning stages (~1250 litres of solvent is used per 1000 litre reactor (Roschangar *et al.*, 2018). To demonstrate the intensities of solvents further, a modular cradle-to-gate life cycle assessment (LCA) of API manufacture was conducted by scientists and engineers at GSK. It was found that volatile organic compounds (VOCs) i.e., solvents were the major impact contributors accounting for 75% of energy use, 80% of total mass of materials, 75% of photochemical oxidation, 50% of greenhouse gas emissions and 10 - 40% of manufacturing cost (Jimenez-Gonzalez, Curzons, Constable and Cunningham, 2004).

2.3 Green Chemistry Philosophy

Greenness embodies a preventative strategy that avoids dealing with the potential consequences of process accidents and mistakes. In 1990 the US environmental protection strategy implemented the "Pollution Prevention Act" to minimize pollution permitted and avoid remediation methods. Preventative green methods thus shift the focus from "end-of-pipe" cleaning strategies (i.e., reduction and neutralization of pollutants before being emitted into the environment) towards "front-of-pipe" cleaner development (i.e., eliminating pollution at the onset of the chemical synthesis) (Cespi, Cucciniello, Ricciardi, Capacchione, Vassura, Passarini and Proto, 2016).

In 1998, the foundation of sustainable chemical design known as the green chemistry philosophy was established by Anastas and Warner (Anastas and Warner, 1998). Green chemistry may be defined as

sophisticated and innovative chemistry that aims to maximize reaction efficiency whilst eliminating or reducing human and environmental harm. It promotes the efficient utilization of raw materials, avoiding the use of hazardous solvents and reagents and pollution prevention with the desired outcome of zero waste production to reduce the cost of waste disposal (Vert, Doi, Hellwich, Hess, Hodge, Kubisa, Rinaudo and Schué, 2012). Green chemistry thus aims to develop inherently safer design of molecules, materials and processes at both a laboratory and industrial setting.

A common 21st century goal across all chemical industries is the contribution towards developing a sustainable future. Sustainability is commonly defined as a development that "meets the needs of present generations without sacrificing the ability for future generations to meet their own needs" (Sheldon, 2017b). Implicit within this definition are the three main pillars of a sustainable process which include economic development, environmental impact and social equity, commonly referred to as the triple bottom line.

Through the use of safe alternative solutions, green chemistry minimizes the impact of earlier industrialization eras whilst simultaneously not compromising the needs of future generations (Cue, 2012). Thus, green chemistry embodies the ideology of sustainability. According to Jiménez-González *et al.*, (2012), green chemistry incorporates the realities of the 21st century by developing products being mindful of the impact on eco-systems and human health, reduction of material cost, toxicity and energy consumption.

Green chemistry may be implemented by chemists and chemical engineers using the 12 principles of green chemistry and engineering. Even though chemical reactions and processes may not be 100% green, their negative impacts may be significantly reduced using the principles as design guidelines. The principles of green chemistry and engineering are intimately linked in promoting safe and minimal waste generating chemical processes (Roschangar, Sheldon and Senanayake, 2015). These principles thus provide a good framework in addressing the pressing need for the pharmaceutical industry to reduce its environmental footprint.

The Environmental Protection Agency (EPA) defines the field of green chemistry as the "design of chemical materials and processes that minimize or eliminate the use or generation of hazardous substances" (United States Environmental Protection Agency, 2006). Green engineering is described as "the design, commercialization and use of products and processes that are feasible and economical while minimizing both the generation of pollution at the source and the risk to human health and environment" (United States Environmental Protection Agency, 2017). These disciplines represent greenness at different stages of a product's life cycle from discovery to process design to commercialization.

Green chemistry helps address the environmental concerns of process design at the most fundamental level i.e., the molecular level. It involves the manipulation of atoms to generate desired products without simultaneously generating hazardous substances (Cue, 2012). The 12 principles of Green Chemistry are the foundation for guiding the sustainable design of new molecules that aim to contribute towards a sustainable future.

The 12 principles of Green Chemistry are listed below (Anastas and Warner, 1998):

1. *Prevention*- More favourable to prevent waste than to clean up waste after it has been formed.
2. *Atom Economy*- Design synthesis routes to maximise the incorporation of all the materials used in the process into the final product.
3. *Less hazardous synthesis*- Design synthesis routes to use and generate substances that cause minimal toxicity to human and environmental health.
4. *Design benign/safer chemicals*- Chemical products should be designed to effect their desired function with minimal toxicity.
5. *Benign/safer solvents and auxiliaries*- solvents and auxiliary substances (e.g. separation agents) should only be used when absolutely necessary in a safe harmless manner.
6. *Design for energy efficiency*- Energy requirements should be minimized due to their economic and environmental impacts. Synthesis processes should use ambient temperatures and pressure wherever possible.
7. *Renewable feedstocks*- Feedstocks should be renewable rather than depleting whenever technically and economically possible.
8. *Reduced derivatives*- Unnecessary derivatization (use of blocking groups, protection/ deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.
9. *Catalysis vs. Stoichiometry*- Selective catalytic reagents are superior to stoichiometric reagents. Catalysis and bio-catalysis are also used in improving the efficiency and conduction of the synthesis reactions.
10. *Design for degradation*- Chemical products should be designed in such a manner that at the end of their functional life, they break down into innocuous degradation products and do not persist in the environment.
11. *Real-time analysis for pollution prevention*- Develop analytical methods that allow for in-process monitoring and control prior to the formation of hazardous chemical compounds.
12. *Inherently benign chemistry for accident prevention*- Substances and their form should be chosen for minimal potential of chemical accidents (e.g. explosions, fires etc.)

Extrapolation of these principles for industrial application are given by the 12 principles of Green Engineering (Anastas and Zimmerman, 2003) and in the 9 Principles of Green Engineering (Abraham

and Nguyen, 2004) listed in Appendix D.1 and D.2 respectively. These principles hold the same core values and general themes as green chemistry principles, however are focused on the sustainable design of products at a pilot or manufacturing scale.

The field of green engineering covers a wider spectrum of a chemical product's life cycle. Typically, green engineering involves the development and use of mathematical tools and metrics that aid in decision making when faced with alternatives, especially when trade off solutions are presented. Green engineering additionally considers 'green technologies' with a higher environmental, safety, quality and economic performance. Some examples of green chemical engineering subject matter include: operations for superior heat transfer and mixing, increasing the safety of hazardous batch chemistry, minimizing batch to batch variation, processes running under steady state control, investigating plug flow reactors (PFR) and continuous stirred tank reactors (CSTR), energy intensive distillation replaced by organic solvent nano - filters and so forth (Koenig, Mergelsberg and Roschangar, 2017).

From an LCA perspective, green chemistry and engineering principles aim to prevent waste at the source as well as improve areas at all stages of the chemical process or product life cycle and hence provide "life cycle innovation" (Anastas and Lankey, 2000). Furthermore, the father of green chemistry Anastas stated that "through a systematic incorporation of green chemistry into the design of the next generation drugs" the pharmaceutical industry will provide products that "throughout its life cycle will benefit human health both for the patient and all others impacted by its manufacture and disposal".

3 Method Development

3.1 Green Metrics

Over the past few years, trends towards sustainable solutions have led to a wide range of initiatives within the chemical industry. Green chemistry principles, metrics and assessment tools provide chemists and chemical engineers with guidelines and measures that aid in developing safer and less impactful products and processes.

Notwithstanding the usefulness of green chemistry principles, their qualitative nature often poses limitation that needs to be supplemented with quantitative measures. The successful implementation of green chemistry lies in translating them into mathematical equations known as green metrics (Andraos and Sayed, 2007). Combining the principles with quantitative measures will support and strengthen environmentally friendly claims as the proverb says, 'you can't manage what you don't measure' thus implying that metrics and tools are of utmost importance in conveying greenness performance (Sheldon, 2017a).

Green metrics are derived from chemical reaction stoichiometry and experimental procedure quantifying environmental performance. A number of these metrics are based on mass balances, obeying the law of conservation of mass, forming the basis of quantitative measures for process assessments. Potential improvement areas using metrics may be identified to increase process greenness at conceptual and early design stages. Commonly used green chemistry metrics with their respective mathematical formulas, optimum values and considered chemistry variables are summarized in Appendix E.2.

Since each green chemistry principle is not clearly defined by metrics, Jiménez-González and Constable (2011) classified the twelve green chemistry principles into metric categories to aid green assessments as illustrated in Table 3. Possible reasons for green chemistry metrics not being routinely implemented within pharmaceutical development is attributed to the pressure of timely drug route design along with the belief that bench scale syntheses are too small to generate significant waste (Cue, 2012).

Table 3:The twelve principles of green chemistry broken down into metric categories (Jiménez-González and Constable, 2011)

Metric category	Green chemistry principle number
Mass efficiency	1, 2, 5, 8, 9
Health and safety	3, 11, 12
Energy efficiency	6
Waste generation	4, 10
Renewability of raw materials	7

As previously described, sustainability is comprised of three main pillars: societal, ecological and economic. Sheldon (2017) describes each pillar of sustainability as one-dimensional defined by its own set of metrics. A sustainable operation entails the integration of all three pillars, making its assessment multidimensional with interlinked metric relations. The combined use of metrics is a powerful tool in evaluating the environmental impact performance of pharmaceutical processes, with methodologies continuing to be refined (Sheldon, 2017b).

The application of a universal set of green metrics to assess material efficiencies of chemical reactions and synthesis plans is well documented (Andraos and Sayed, 2007; Beach, Zheng and Anastas, 2009; Mercer, Andraos and Jessop, 2011; Dach, Song, Roschangar, Samstag and Senanayake, 2012; Roschangar, Sheldon and Senanayake, 2015; Roschangar, Colberg, Dunn, Gallou, Hayler, Koenig, Kopach, Leahy, Mergelsberg, Tucker, Sheldon and Senanayake, 2017). These metrics are however inconsistently used in literature. To eliminate confusion of metrics used within the pharmaceutical industry, Roschangar, Sheldon and Senanayake, (2015) created a graphical illustration of green mass metrics used in realtion to chemical material categories, as illustrated in Figure 5.

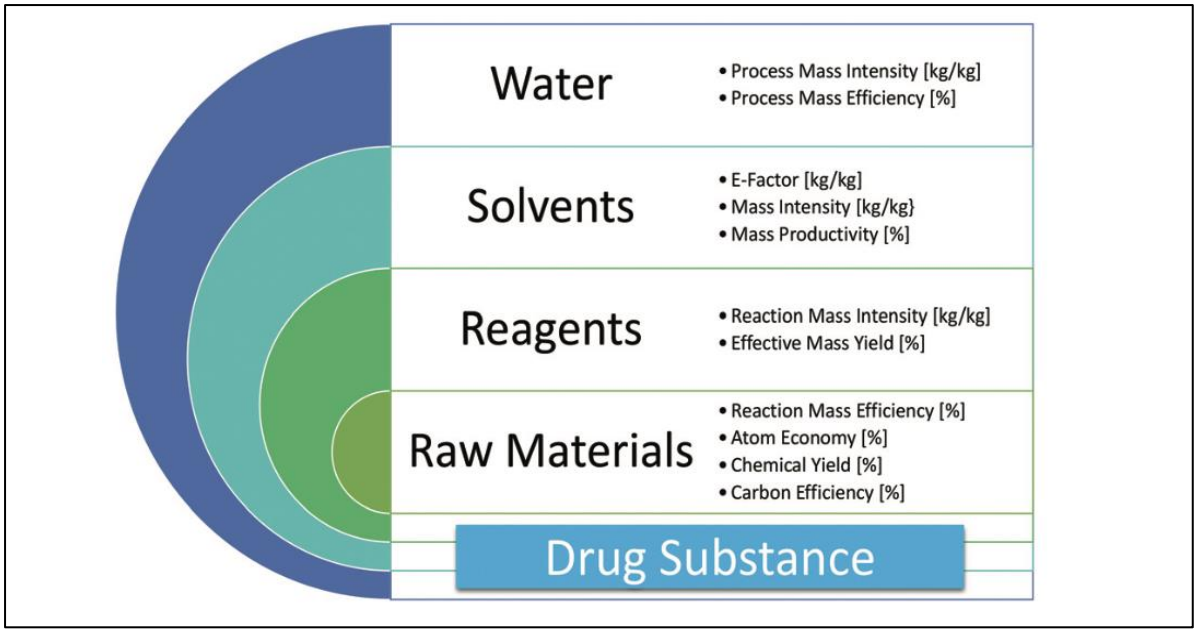


Figure 5: Relationship between green metrics and chemical materials (Roschangar, Sheldon and Senanayake, 2015)

From a process economics perspective, reaction yield, quantity of reagent and solvent materials used have the greatest influence. Further, Constable, Curzons and Cunninghama, (2002) reported that > 75% of material costs are accredited as waste materials (i.e., reagents and solvents that do not form part of the target molecule). Even though green metrics atom economy (AE) and carbon efficiency/economy (CE) are of less economic importance, they still aid in demonstrating the demerits of high molecular weight reactants and protecting groups that indeed contribute towards high waste generation (Constable, Curzons and Cunninghama, 2002).

As illustrated in Figure 5, AE and CE are the metrics used in considering raw materials and the final drug compound. AE is a conversion efficiency metric that determines the amount of atoms of reactants (usually only two) that are present in the final product (Constable, Curzons and Cunninghama, 2002). Similarly, CE considers the number of carbon atoms in the reactants that are present in the final product. The CE metric thus follows a similar trend to AE (Phan, Gallardo and Mane, 2015). Ideal AE scores are created using raw materials with low molecular weights that form part of the desired product. The formula for AE considers the molecular weights of the raw materials and product compounds whereas the CE considers only the carbon atoms of the raw material and product compounds (Phan, Gallardo and Mane, 2015). For both metrics additional reagents, inorganic reactants, solvents and catalysts are ignored.

Along with AE being introduced in the early 1990's was Sheldon's E-factor. These two metrics were established before the Green Chemistry movement and for the past two decades have been widely used

in both academia and industry (Sheldon, 2017a). The E-factor however, remains the most well-known and widely used mass metric in quantifying the environmental impacts of a chemical process (Sheldon, 1992). It measures the amount of waste generated within a chemical process, where waste is defined as all chemical materials other than the desired product (i.e., waste = raw materials – desired product) (Brady, 2019). The E-factor only relates to the chemical process carried out at the manufacturing site and thus from a life-cycle perspective works on a gate-to-gate basis (Sheldon, 2018b).

Further, the E-factor is highly dependent on the starting point of the synthetic procedure. Commonly, the starting materials used at pharmaceutical manufacturing sites are advanced starting materials (ASM's) prepared by multi-step synthesis with their own E-factor. As defined by Roschangar *et al.*, (2018) the ASM is identified by being unavailable as a commodity material or having a cost of over $100/mol, for the largest quantity available from a reputable commercial supplier. The E-factor associated with the ASM synthesis is known as the intrinsic E-factor. Neglecting the intrinsic E-factor will lead to skewed measure of the true waste generation of the complete synthetic pathway to the desired API. Thus, the intrinsic E-factors needs to be accounted for to provide a common and fair starting point and prevent inconsistencies in measuring the environmental impact of an entire synthesis route. Since the E-factor metric is additive, the intrinsic E-factors may be simply added to the main synthesis E-factors to obtain an unbiased E-factor value for a complete synthetic pathway to a desired product (Roschangar, Sheldon and Senanayake, 2015) .

Moreover, in 1992 Roger Sheldon published an inventory of E-factors across a range of chemical sectors as previously illustrated and discussed in Table 2. This table is used as a benchmark for the waste produced in chemical industries and provides a challenge specifically to the pharmaceutical industry to reduce the amount of waste generated in their manufacturing operations.

The 'classical' or 'traditional' E-factor is defined as the 'mass ratio of waste to desired product' that considers reagents, solvent losses (assumes 90% solvent recovery) and no process water as mathematically represented in Equation E1. Water is omitted from the calculations as it has a negligible contribution towards environmental impact and skews the mass data (Constable, Curzons and Cunningham, 2002). The E-factor metric considers the stoichiometry, yield and waste to quantify the environmental impact of a chemical processes.

$$E - factor = \frac{\Sigma \mathrm{m(Raw\ materials)} + \Sigma \mathrm{m(Reagents)} + 0.1\Sigma \mathrm{m(Solvents)} - \mathrm{m(Product)}}{\mathrm{m(Product)}} \frac{[kg]}{[kg]} \qquad (E1)$$

The optimum E-factor value is zero. High E-factor values corresponding to more waste generated and hence greater environmental impact. Lower E-factor values are directly related to smaller quantities of materials used and show a strong positive correlation with reduced manufacturing and waste disposal costs.

The original E-factor exhibits a number of limitations including:

a. Process water was not included because including it would, it was thought, skew the E-factors.
b. Not considering the nature of the waste, assigning all waste types were given the same weighting.
c. Waste derived from energy use was not included because it was not easy to allocate to products in multi-purpose facilities although it was implicitly assumed to contribute as kgs CO_2 per kg fuel consumed.

To combat these limitations, the E-factor has been refined into new respective derivatives:

a. The simple(s) and complete(c) E-factor:
b. The Environmental Quotient (EQ)
c. The E^+- factor (Tieves, Tonin, Ferna ndez-Fueyo, Robbins, Bommarius, Bommarius, Alcalde and Hollmann, 2019)

The sE-factor metric does not account for water or solvents and is commonly used in the early development stage of the synthetic pathways. From the pharmaceutical timeline perspective this metric is used prior to clinical trials phase 1 (Pharmaceutical Timeline, Section 2.11). Conversely, the cE-factor metric incorporates all process materials (i.e., raw materials, reagents, solvents and water) with no recycling of solvents or water considered and useful at the post finalization or phase 2 of clinical trials of the pharmaceutical timeline (Roschangar, Sheldon and Senanayake, 2015). The cE-factor follows the current trend in the pharmaceutical industry by accounting for process water which is important to consider given the stress placed on the scarcity of water resources. It may however be argued that water has a negligible environmental impact and thus should not be considered as a waste material. However, since water usage was benchmarked at 32% of pharmaceutical process material mass, its cost should not be neglected (ACS Green Chemistry Institute, 2014).

The traditional E-factor value of a process lies between the sE-factor and the cE-factor value, which may be calculated when solvent and process water recycling estimates are known (Roschangar, Sheldon and Senanayake, 2015). If the solvent losses are not specified then a solvent recovery of 90% is assumed (Sheldon, 2017a). This is also referred to as the “recycling-adjusted E-factor”.

The EQ-factor considers the mass of the material as well as the nature of its environmental burden in terms of hazard and depleting resources. The weighting of Q is qualitative and based on monetary treatments and environmental laws (Sheldon, 2017b).

The E^+- factor considers the GHG emissions generated from electricity used for cooling, heating, stirring, pumping etc. (Tieves, Tonin, Ferna ndez-Fueyo, Robbins, Bommarius, Bommarius, Alcalde

and Hollmann, 2019).

Moreover, the E-factor metric is related to Process Mass intensity (PMI) as illustrated by Equation E2,

$$E - factor = \text{PMI} - 1\ \frac{[kg]}{[kg]} \qquad (E2)$$

This slight difference in mathematical formula allows for the shift of perspective when communicating process greenness. The E-factor returns a numerical outcome for waste generation whereas PMI returns a numerical outcome for material use. The ACS GCIPR makes use of the PMI metric to communicate the material impacts in API manufacture. However, unlike PMI the E-factor is an additive metric which makes it easier to assess multi-step synthetic procedure (Roschangar, Sheldon and Senanayake, 2015). Further, "waste generation" are the key words in communicating the environmental impact status within the pharmaceutical sector. According to Sheldon (2012), "none of the alternative green mass metrics offer great advantage over the E-factor for describing how wasteful a process is." This is particularly true when considering that waste elimination has always been the major driving force behind the green chemistry movement. For the purpose of this dissertation, the E-factor metric will be used in assessing the wastefulness of the selected synthetic pathways to the bis-THF alcohol.

The merits of green metrics lie in their simplicity, ease of use and ability to provide instant feedback on a chemical procedure. This allows for modelling of and changes in the process or synthesis route to be easily implemented (Tufvesson, Tufvesson, Woodley and Börjesson, 2013). Conversely, a significant demerit of these metrics is the assignment of one environmental impact category (e.g., waste generation) for all metric output values. This is due to the oversimplified assumption that materials not forming part of the desired final product are considered as waste. Consequently, the "multi-dimensional nature of the environmental problems" might not be considered which poses a serious risk of these metrics sub-optimizing the chemical procedure (Tufvesson, Tufvesson, Woodley and Börjesson, 2013). Thus, green metric analysis based solely on material efficiency is an inadequate basis in comparing the greenness of synthesis routes since the nature of the substances and energy factors of the process should also be considered (Mercer, Andraos and Jessop, 2011). It is thus essential to incorporate additional green chemistry guides and tools for a more holistic environmental impact assessment of chemical syntheses and processes.

3.2 Early Stage Assessment Tools

Currently trending are sustainable chemistry internet tools that aim at quantifying the greenness of chemical synthesis and processes at conceptual or early design stages. Common early stage assessment tools are web-based guides or simple computer models that are based on multiple input variables requiring materials mass, energy (from reported experimental procedures) or safety information found in literature. These tools aim to provide sustainability analysis based on a portion or all of the twelve

principles of Green Chemistry and Engineering to provide a more holistic perspective on the multi-dimensional environmental impacts of the chemical operations (Cue, 2012). More specific examples of the information required in green tools include: reaction yields, hazard and toxicity status of materials, renewability of starting materials, type of energy used, reaction time and temperature, catalysts and solvents required to perform the reaction and/or purification or separation operations (Mercer, Andraos and Jessop, 2011). Generally, these web-based tools and models report on process efficiency (material consumption), energy efficiency (energy consumption), resource efficiency (petrochemical, non-renewable or bio-based renewable raw materials), environmental impacts and performance versus industrial standards for chemical processes (Andraos, 2012).

Numerous tools have been developed to inform and assist chemists and chemical engineers in practicing and implementing green chemistry. For instance, web-based reagent guides created by the ACS GCIPR were designed to help chemists select ideal reagents for API synthesis. The guideline makes use of Venn diagrams that consider the 'scalability', 'broad utility' and 'greenness' of reagents used in the required chemical transformation (ACS Green Chemistry Institute, 2015). Additionally, since the vast majority of chemical reactions are conducted in large solvent volumes, solvent guides have been published to aid chemists in their solvent selection process (details in Section 2.7 and 3.3).

Examples of other web-based tools and software programs available to assess environmental impacts include, Eco-solvent, Fine Chem, SimaPro, Gabi and OpenLCA to name a few (ETH Zurich, 2008; The Ecoinvent centre, 2010; PRé Consultants: Amersfoort (NL), 2017). Numerous papers have reported the successful use of such software in assessing the relative efficiency and greenness of alternative synthesis routes to a specific target molecule (Rosini, Borzatta, Paoluccia and Righi, 2008; Kinen, Rossi and de RossI, 2009; Protti, Dondi, Fagnonia and Albini, 2009; Ravelli, Protti, Neri, Fagnonia and Albini, 2011; Ribeiro and Machado, 2011; Werner, Machara, Sullivan, Carrera, Moser, Adams and Hudlicky, 2011; Toniolo, Fabio Arico and Tundo, 2014; Zhang, Gitungo, Axe, Dyksen and Raczko, 2016; Sevenich, Liu, Arduengo III, Gupton and Opatz, 2017). Some of this software is focused on one environmental category. For example, Eco-solvent specifically focuses on the environmental impact of solvent waste. Hence, a more holistic assessment of the synthetic pathways requires the use of more than one software (Capello, Hellweg and Hungerbühler, 2007 cited in Toniolo, Fabio Arico and Tundo, 2014).

As illustrated in Figure 6, environmnetal assessments using LCA are only conducted at 'implementation and marketing' stages where there is greater availability of process data and lower risk of change in process design. Environmental assessments using green metrics and metric based tools are considered during the conceptual design stage, where there are less process details, high degree of process uncertainty allowing for a greater influence on the innovative process design. It is at the conceptual stage that numerous potential synthetic procedures may be compared (Tufvesson *et al.*, 2013).

Environmental assessments using 'simple models' are considered during the process development stages, where there is moderate availability of process details, a certain degree of process uncertainty and reasonable influence on process design. The intensity of the assessments performed on the chemical procedure is thus dependent on its development stage. The larger and more developed the chemical procedure, the more data are available and the greater the complexity of the assessments performed.

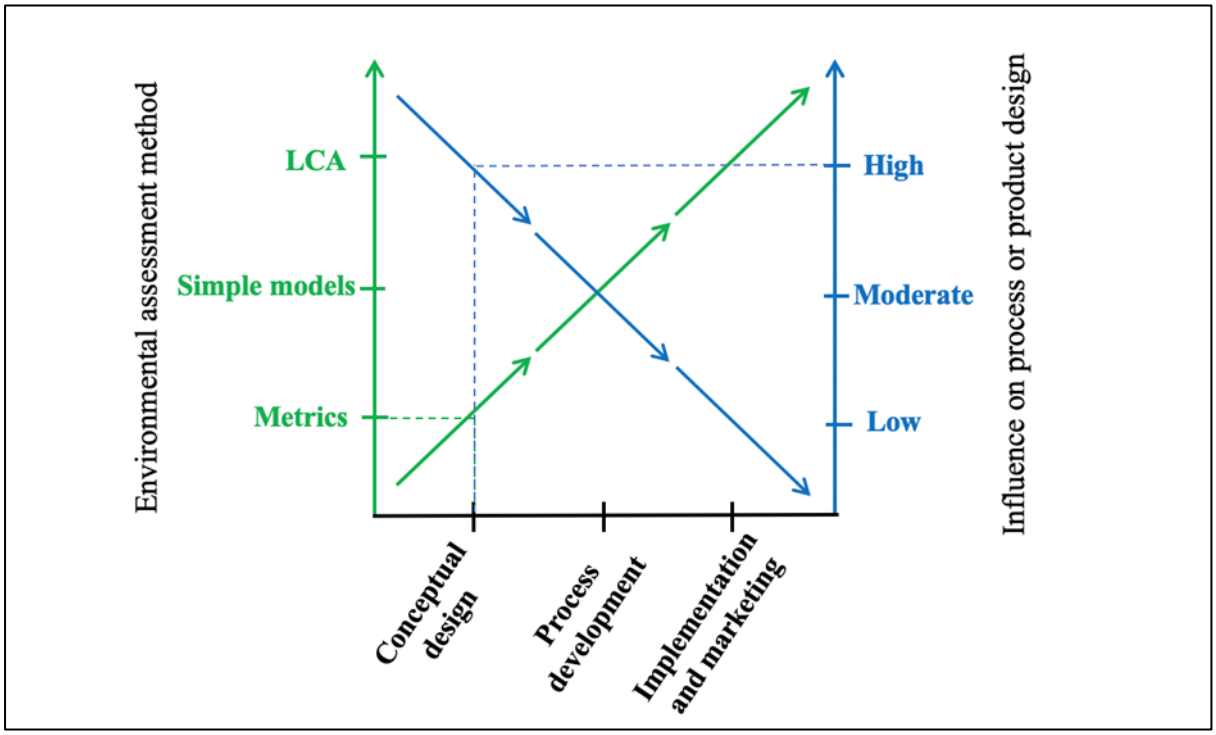


Figure 6: Environmental assessment methods and their level of influence at different development stages of the chemical process (as adapted from Tufvesson et al., 2013).

Examples of web-based tools and guides that are applicable at a conceptual design stage used in assessing the greenness of the bis-THF alcohol include Green Motion™, innovative Green Aspiration Level (iGAL™) green scorecards and the GSK solvent sustainability guide. It is also important to note that the E-factor metric and its variations were incorporated into the Green Motion™ and iGAL™ green scorecards assessment tools. Section 2.6 through 2.9 describe the web-based tools, guides and comparative assessments that were used to assess the environmental impacts of the bis-THF alcohol.

3.3 Green Scorecards - iGAL™

The innovative Green Aspiration Level (iGAL™) methodology, created by the joint efforts of the ACS GCIPR and academia (Sheldon), is an improved measure of process greenness from the original Green Aspiration Level (GAL™) methodology (Roschangar et al., 2018). The aim of iGAL™ is to innovate sustainable API synthesis design and to overcome the limitations associated with waste metric consensus through a 'unified' green chemistry metric. iGAL™ is thus a performance metric that captures the inventiveness in reducing environmental mass impacts in API synthesis, relative to

industrial standards. The iGAL™ along with the cE-factor metric are graphically represented on a Green Scorecard and demonstrate the waste reduction (mass-efficiency) of the synthesis at different development stages, relative to industrial benchmarks (Roschangar, Sheldon and Senanayake, 2015). Future prospects of the iGAL™ and Green Scorecard are to incorporate the correlation between waste reduction and process economics (to motivate a strong business case for process greenness) as well as LCA assessments (Roschangar et al., 2018).

3.4 Solvents

Solvents play a critical role across chemical industries with millions of tons used and disposed of each year. Specific to the pharmaceutical industry solvents contribute towards 80 - 90% of the total non-aqueous material mass and 75 - 80% of the environmental life cycle impacts in synthesizing API's (Constable, Jimenez-Gonzalez and Henderson, 2007 and Jimenez-Gonzailez *et al.*, 2004). This is attributed to solvents being multi-functional in formulating API's for example, providing the reaction medium (mass transportation and mixing), extraction and purification medium as well as cleaning of reactors (Koenig, Mergelsberg and Roschangar, 2017 and American Chemistry Council, 2019). Solvents are thus responsible for substantial amounts of waste formed in API manufacture.

It is common practice for chemists to make excess use of undesired solvents. This creates a series of environmental and human health issues including global warming, greenhouse effect, destruction of the ozone layer and toxicity (Vafaeezadeh and Hashemi, 2015). For example, solvents such as diethyl ether, dichloromethane, benzene and aliphatic hydrocarbons are commonly used solvents in the history of organic synthesis that are now classified as hazardous with restricted use by legislation (Sheldon, 2019). Green solvents are not easily defined as they are dependent on multiple performance criteria including: health and safety status, biodegradability, derivation from renewable feedstocks, ease of recycling, low flammability and energy demand, insoluble in water, moderate toxicity and reduced cost contribution to organic synthetic procedures (Koenig, Mergelsberg and Roschangar, 2017). Implementing the use of less hazardous solvents at early drug discovery phase increases the probability of saving time, money and adhering to environmental regulations before API synthetic pathways are locked and scaled up to commercial stages.

3.5 Green Motion™

Green Motion™ is a web-based metric tool created in 2015 by MANE, a world leading flavor and fragrance manufacture. It is freely available on the MANE website to aid chemists in pursuing their commitment to social responsibility by developing greener solutions. It provides a well-rounded assessment on process greenness based on seven fundamental concepts that are derived from the twelve principles of green chemistry. MANE has included the Green Motion™ rating tool as a key selection

criterion, in combination with process costing and performance, in manufacturing their products and has rated the environmental impacts of over one thousand chemical ingredients.

The Green Motion™ tool is described as a simple and multi-criteria methodology that operates like an "audit grid" (Phan, Gallardo and Mane, 2015). From an LCA perspective, the tool is a "gate-to-gate" assessment that considers the manufacturing stage of the LCA (Phan, Gallardo and Mane, 2015). It is important to note that LCA tools are of far greater complexity and more comprehensive in assessing environmental impact categories and performance, however these assessments require large amounts of inventory data that may not be available for specific products and processes. Further, such intensive assessments may not be relevant or clear at conceptual or early design stages.

3.6 Radial Polygon

The radial polygon provides a concise visual compilation of green metric results, an example of which is illustrated in Figure 7. Each vertex of the polygon (usually pentagon or hexagon or octagon) represents an ideal metric value and the centre of the polygon the least ideal value (Andraos and Sayed, 2007). The ideal green synthesis is depicted by the regular polygon. Each synthesis route will be represented by a distorted polygon constructed by connecting the route's marked parameter value on the radius to each vertex. The less "green" the synthesis route, the greater the distortion of the regular polygon towards the centre, directly linking the parameter responsible for the distortion (Vanden Eynde, 2016). This will allow for rapid identification of each synthesis routes weakness parameter (Vanden Eynde, 2016), guiding synthetic chemists in determining which areas of the experimental procedure need to be adjusted and consequently resulting in an environmentally friendlier synthesis.

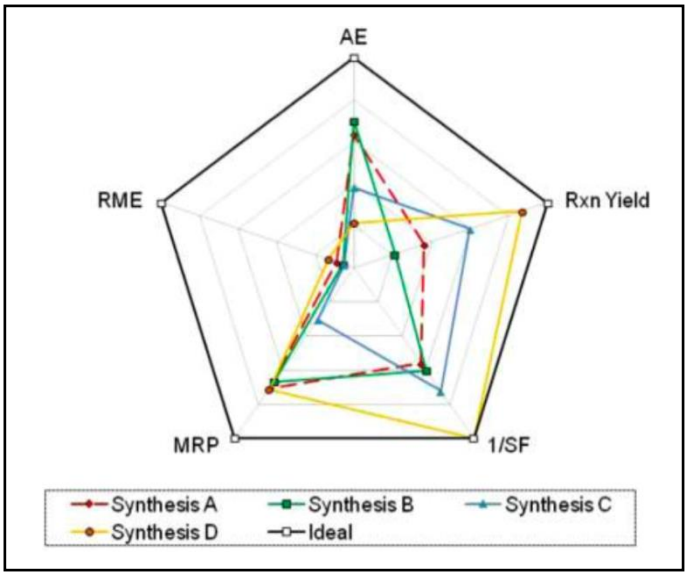


Figure 7: Radial pentagon comparing raw material footprints of four synthesis routes to a target molecule (Toniolo, Fabio Arico and Tundo, 2014).

3.7 Life Cycle Assessments

LCA is a methodology adopted by industries, academia and government institutions to quantify a product's or process's entire environmental impact (starting from raw material production, manufacturing and product disposal i.e., cradle to grave). The LCA is significant in industrial sectors in identifying which areas of a process need to be improved for environmental benefit. The principles and methodological framework of LCA are well described in the International Organization for Standardization (ISO) 14040 (2006). The standard emphasizes the importance of a clearly defined goal and scope, collection of all necessary input data which is "processed" in an impact assessment the outcome of which is subsequently interpreted and evaluated (Tufvesson *et al.*, 2013). Important factors considered include costs, safety, atmospheric emissions, environmental risks etc., preceding and following the synthetic manufacture of the chemical product. Gabi and OpenLCA are a licensed LCA software which may be used to assess environmental impacts of products and processes within the specified boundary conditions (gate-to-gate, cradle-to-gate, gate-to-grave, cradle-to-grave).

The value in using LCA for comparative assessments between conventional and "green" chemical processes has been documented (Anastas and Lankey, 2000; Azapagic, 2002). LCA however demands the need for extensive input data (inventory data) that is easily available for well-established chemical products and processes, especially those of fossil-based platform chemicals. Conversely, it is a great challenge to obtain the extensive data for chemical processes at research or early development stage, especially when involving high chemical complexity (most pharmaceuticals), biotechnologies (such as biocatalysts) and renewable resources (with the exception of biofuels) (Tufvesson, Tufvesson, Woodley and Börjesson, 2013). Without accurate extensive data, conducting a complete LCA is deemed inaccurate.

More specifically for medicinal products, complexities arise in broadening the boundary conditions of the LCA and considering the after use and emissions of pharmaceuticals predominantly occurring in river and lakes (Wernet, Conradt, Isenring, Jiménez-González and Hungerbühler, 2010). A point of contention lies in setting the boundary conditions for the "cradle" and "grave" limits of a pharmaceutical LCA. Tufvesson *et al.*, (2013) stated that cradle-to-grave assessments are more easily conducted for bulk chemicals and cradle-to-factory gate for fine chemicals and pharmaceuticals. This is attributed to the fact that these types of specialized chemicals have different end-of life scenarios. System boundaries suitable for 'green chemistry' comparisons of different production routes for manufacturing a common medicinal target compound, are cradle-to- gate or gate-to -gate (Klopffer, 2005 cited in Tufvesson *et al.*, 2013).

The inventories required for the synthetic pathway LCA assessment would be obtained from the experimental data and where information is missing from other databases (e.g., Eco-invent). The

application of LCA assessments at a synthetic stage may however be unnecessary as it is too detailed and extends beyond the knowledge of synthetic chemists. Moreover, GSK scientists and engineers concluded that LCA is not widely practiced within the pharmaceutical industry owing to the limited inventory data available for materials routinely used in API synthesis. Despite this, it was reported that solvents were the greatest contributors in most impact categories for API manufacture as previously discussed (Jimenez-Gonzalez, Curzons, Constable and Cunningham, 2004).

More commonly practiced within the pharmaceutical industry is life cycle thinking or life cycle awareness. These approaches require the use of quantitative data (obtained from green metrics and tools) and qualitative data (risk assessment and MSDS data) to simply and clearly communicate impacts. Even though much less comprehensive than full LCA, this type of thinking aims to broaden perspectives in trying to understand the impacts associated with material and energy selections, health and safety risks and waste generation which are important in early stage synthesis design. Life cycle thinking is simply gaining insight into the environmental impacts of pharmaceutical activities within a reasonable time frame.

Moreover, fine chemical and pharmaceutical companies, such as BASF and GSK, respectively, have developed unique *in house* LCA methods, based on ISO guidelines, to assess and evaluate the sustainability of their chemical processes (Curzons *et al.*, 2007). Srinivas (2016), boldly stated that chemical companies are undergoing a "trend of more open disclosure of environmental information" and that consumers are gaining the "desire" to be "guided towards the least harmful purchases", making environmental assessment tools of vital importance at early stages of chemical process development. More specifically, GSK designed and developed Fast Life Cycle Assessment for Synthetic Chemistry (FLASC™) in alignment with Eco-Design Toolkit®; a tool used to quantify the mass and energy utilization of the pharmaceutical processes. FLASC™ investigates the environmental impacts of the pharmaceutical process using a cradle-to-gate life cycle assessment. The software's usefulness lies in rapidly processing and comparing chemical syntheses to a desired target molecule assisting scientists and engineers to meet short deadlines within stringent environmental regulations thus maintaining sustainable business (Curzo ns *et al.*, 2007). FLASC™ database has also been used in conducting an LCA on enzymes for application in pharmaceutical products (Kim, Jiménez-González and Dale, 2009). Additionally, BASF developed "Eco-efficiency analysis" tool and methodology that identifies the most economically efficient manner in solving environmental process issues (Tufvesson, Tufvesson, Woodley and Börjesson, 2013). These tools are not however freely available to the public.

3.8 The Pharmaceutical Timelines

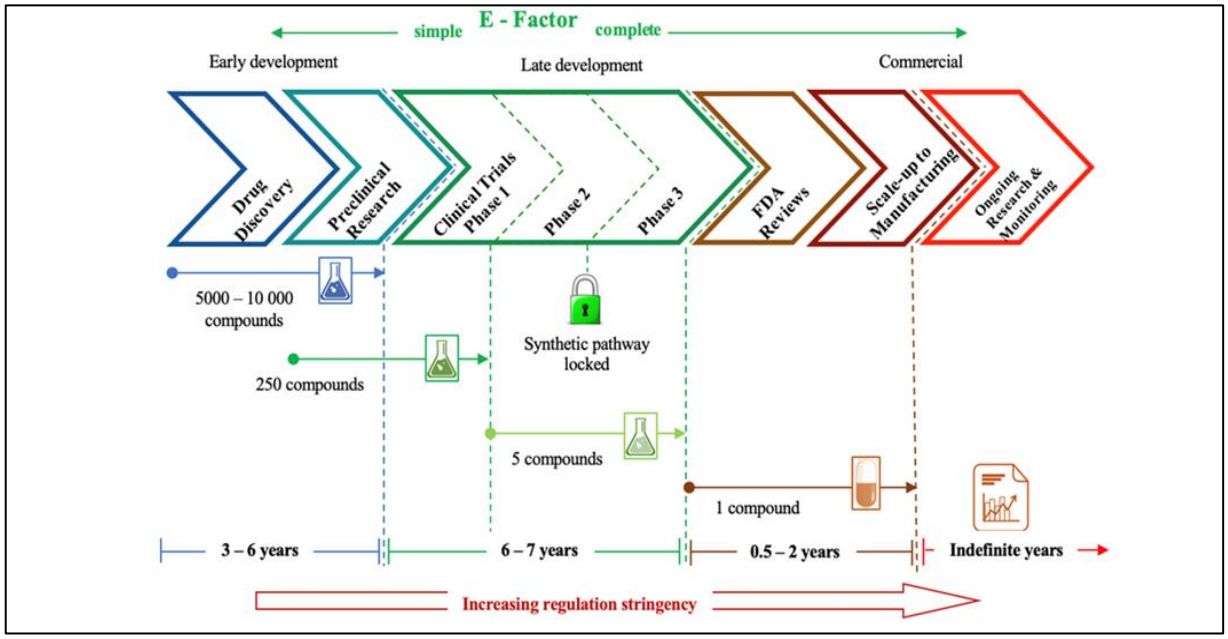


Figure 8: Timelines and development stages of pharmaceutical compounds (as composed from GSK 2017; Gaffney, Mezher and Brennan 2017 and Roschangar, Sheldon and Senanayake 2015).

At the discovery stage, medicinal chemists find target molecules that may treat a disease, weaken an invasive viral or bacterial organism or influence metabolic processes. The target molecule then enters the design and development stage and is assigned to the chemist to design an efficient synthetic pathway to the compound (Jiménez-González and Constable, 2011). The synthetic chemist is limited to the exact design of the target molecule and may not change the molecule's functional groups, spatial orientation or add any other chemical constituents to the molecule. This is because the specific function of the target molecule has already been determined. At this stage, the medicinal chemists perform a retrosynthesis[6] to find starting materials and efficient reaction sequences to the target molecule. Thereafter, appropriate reagents, solvents, catalysts and their quantities are selected along with reaction conditions. During this stage, chemists are more concerned with the feasibility of using a particular synthesis to produce enough of the target molecule for (pre)clinical trials within a certain time frame. The cost and environmental impact are less important at this stage.

[6] Retrosynthesis is a technique used by chemists in devising a synthetic pathway to the target molecule. This entails working backwards from the target molecule and systematically dissecting the molecule by means of strategic disconnections. This leads to the identification of smaller or commercially available molecules that may be used as the starting material. Chemists may then use *functional group interconversions* where well - known reactions are identified and potentially used to obtain the target molecule. The synthetic pathway to the target molecule is simply the reverse of the retrosynthetic analysis (Jiménez-González and Constable, 2011).

Figure 8, illustrates the development stages and respective time frames associated with API manufacture. Considering all stages, it may take medicinal compounds between 9 and 15 years to progress from laboratory to patient. The long time-frame is attributed to the numerous and thorough evaluation processes that need to be conducted before the medication is distributed. A brief description of the pharmaceutical timeline is described below as adapted from (GSK, 2017) unless otherwise specified.

Before pharmaceutical research begins, a disease-causing biological target is identified after which 10 000 chemical compounds are screened to find those effective in combating the undesired biological target. The number of compounds is then reduced to a few hundred based on improved performance (i.e., increased safety of absorption, increased half-life in the body, stable manufacturing, convenient use, minimal known side effects etc.) During the pre-clinical stages, the best performing compounds are tested on cell cultures or animals to better understand their working mechanism. After favourable compound behaviour has been identified the compounds progress to clinical trials. These trials involve the medicinal compounds to be tested on humans. Before testing commences detailed documentation is compiled by an ethical review committee of experienced scientific and medical practitioners. The clinical trials comprise of three phases namely (Smith, 2015): Phase 1- data captured on the pharmacokinetics and pharmacodynamics of the drug compound tested on ~ 15 human volunteers, phase 2- data captured on the dosage concentrations and unwanted side effects on ~150 human volunteers and phase 3- data captured on the therapeutic effectiveness, rare and allergic side effects on $>$ 2000 human volunteers. From a synthesis design perspective, an 80% reduction in solvent mass and synthesis steps should be experienced transitioning between phase 1 and 2 of clinical trials (Koenig, Mergelsberg and Roschangar, 2017). After the medicinal compound has successfully passed the clinical trials, its documentation is submitted for approval to government regulators such as the FDA. This process may take between 0.5 and 2 year to complete as the medicinal compounds efficacy, side effects and good manufacturing practice (GMP) are authorized. After FDA approval the drug has an approximate patent life of 12 years (Vernon, Golec and DiMasi, 2010). During this time, the pharmaceutical company needs to recover all the costs associated with research and development (R&D) of approved and failed drug trials. The estimated recovery is between $1.2 - 1.8 billion dollars and commonly recuperated through sales (Steven, Mytelka, Dunwiddie, Persinger, Munos, Lindborg and Schacht, 2010). After the FDA reviews are completed the medication may then be manufactured at large scale and distributed to doctors to prescribe to patients. Doctors are provided with the required details on the new medicine such that the medication is not prescribed incorrectly. After the medication has reached the market, its long-term side effects and performance under various circumstances are continually assessed and monitored in both humans and animals. This long-term testing may affect the status of the medication over time. The South African Health Products Authority (SAHPRA) is the regulatory authority responsible for the licensing of API manufacturing, control of clinical trials and

distributing of medicinal products in South Africa. SAHPRA follows the "Medicines Act 1995" to ensure that efficient and ethical monitoring, regulation and investigation of API's are conducted (South African Health Products Regulatory Authority, 2020).

Owing to the high demand and short time frame assigned to designing API synthetic pathways (between preclinical and phase 2 clinical trials), pharmaceutical industries do not always consider the best possible molecular assembly strategies to the desired API. As the drug progresses through the development stages, fewer manipulations to the API's synthetic pathway may be made as this may affect the efficacy and quality of the API and hence regulatory requirements become more stringent.

The highest variance in process design occurs between the preclinical and the phase 2 clinical stages where there is little information and knowledge on the synthesis design and minimal impact on regulations. At the end of phase 2, the synthetic design becomes 'locked' with regulations being updated on synthesis details and control information. Any process changes after phase 2 may affect the safety and efficacy of the drug and are thus subject to high risk. In other words, modifications of synthesis design after phase 2 may lead to changes in the drug compound's purity profile (*ee%* and chirality) which could change its biological performance. Synthesis design changes after phase 2 thus require re-validation and approval by the FDA before distribution. Thus, pharmaceutical companies are encouraged to implement all manufacturing and design improvements prior to the phase 2 development stage to avoid time-consuming re-validation of synthetic processes.

Greenness within the pharmaceutical timeline spans over numerous topics from implementing green chemistry at drug discovery to greening process chemistry, work-up procedures and analytical methods. Implementing green chemistry at the onset of pharmaceutical research may avoid the need for synthesis re-design at later development stages. Further, applying green chemistry at early stage design may also benefit the pharmaceutical manufacturing environmentally, economically and socially. This is owing to green chemistry inherently being concerned with the waste reduction, health hazards, toxicity and energy usage in the design and development of chemical products and processes using a set of 12 principles (Cue, 2012). It thus simultaneously acknowledges the protection of scarce non-renewable resources, the restoration of ecological systems as well as cost effectiveness of chemical procedures.

From an economic perspective green chemistry is often combined with the term "more for less" implying more financial returns for less material and energy consumption as well as reduced waste disposal costs (Hawker, 2009). Green chemistry and engineering play a vital role in influencing more sophisticated pharmaceutical research and manufacturing methods. It supports medical innovation, human and environmental safety and aids the production of affordable medicines through the correct selection and evaluation of chemical materials, energy and waste disposal (FDA, 2016).

Green metrics are used to quantify performance greenness of a chemical process. It is difficult to manage and to claim environmental benefits of a process without measurements to substantiate such claims (Meadows, 1998 in Roschangar, Sheldon and Senanayake, 2015). For instance, lower E-factor values directly correlate to reduced material usages and process costs (Leahy, Tucker, Mergelsberg, Dunn, Kopach and Purohit, 2013). Depending on the stage of pharmaceutical development, different refinements of the E-Factor metric may be used (as previously discussed in Green Metrics, Section 2.4).

Green chemistry principles, metrics and tools thus aid chemists in identifying the safest materials and energy methods in designing chemical operations as well as assessing API synthesis greenness. Integrating these assessment techniques as early as the drug discovery phase provides qualitative and quantitative greenness measures, paving the pathway towards sustainable pharmaceutical processes. At the late development stages the ideal synthetic pathway may be optimized using LCA's (Koenig and O'Brien, 2019).

4 Methodology

4.1 Chemical Schemes and E-factor (Excel Spreadsheets).

4.1.1 Explaining the method and design of excel spreadsheets.

Excel spreadsheets A, B and C document the experimental and synthetic details for route A (Hayashi, Aikawa, Shimasaki, Okamoto, Tomioka, Miki, Takeda and Ikemoto, 2016), route B (Sevenich, Liu, Arduengo III, Gupton and Opatz, 2017) and route C (Moore, Stringham, Teager and Yue, 2017) respectively. The purpose of generating the excel spreadsheets was to collect the chemical materials and mass quantities used in each experimental procedure and to manually calculate the E-factor, cE-factor, sE-factor, PMI, iGAL™ and other green chemistry metrics for each synthetic pathway. The recorded chemical parameters and calculated metric values were used in the Green Chemistry Innovation Scorecards and Green Motion™ assessment tools.

Since it is not common practice for chemists to record energy usage at laboratory scale, the energy data was not obtained. Wender, Croatta and Witulskib, (2006) reported that the energy consumption at the manufacturing API stage is minimal compared to the energy requirements used in producing the input materials (i.e., the supply chain energy), thus making the energy data of minimal importance at early synthesis assessment stages. Despite this, the types of energy methods used in each synthetic pathway was accounted for as required by the Green Motion™ assessment.

4.1.2 Rules used in generating excel spreadsheets from experimental procedures.

The experimental data values (mass, volumes, molarity, normality, assay, step yields etc.) for each synthetic pathway were obtained from the respective literature publications and tabulated in Microsoft Excel®. The chemical material properties, MSDS, EHS categories and costs were obtained from various sources, including PubChem and Sigma-Aldrich catalogue respectively unless otherwise specified. All additional calculations were done in Microsoft Excel® version 16.17 and were documented as illustrated in Appendix L. Each spreadsheet contains a schematic representation of the synthetic pathway assessed. Each chemical scheme was drawn using Chem Draw® Prime version 17.1.0.105 and the canonical SMILES code available from PubChem.

The starting materials of the synthetic pathways were identified as compounds that contribute towards the final API structure and are commercially available from a reputable company at the largest quantity available not exceeding $100/mol (Roschangar *et al.*, 2018). The starting materials that were not commercially available and/or did not meet the ≤ $100/mol pricing criteria were categorized as advanced starting materials (ASM). Synthetic pathways to the ASM's were then devised from commercially available starting materials (≤ $100/mol) obtained from a reputable commercial supplier (Sigma-Aldrich catalogue February 2019, unless otherwise stated). The ASM synthetic pathway was

labeled as ‘sub-synthesis’ and its respectively calculated E-factor as ‘intrinsic E-factor’. The intrinsic E-factor was then added to the main synthesis E-factor to achieve the over-all synthesis E-factor value.

Each synthesis step was either classified as a concession or construction step depending on the type of chemical transformation taking place and its contribution to the structure of the target molecule (classification details illustrated in Appendix E.1). This was done to determine the overall synthesis complexity (i.e., Σ construction steps) which was required in the iGAL^{TM} methodology to compare synthetic performance relative to industrial benchmarks. Typically construction steps contribute to the streo-chemistry and functionality of the target molecule (Gaich and Baran, 2010).

Further, each chemical material reported in the experimental procedure was classified into a material category as described in Table 4. The classification of each material is illustrated in each of the Excel spreadsheets and in Appendix L. It is important to note that some chemical materials could be classified in multiple material categories. For example, DMF is commonly categorized as an undesired aprotic polar solvent however in route C may be classified as a solvent, reagent and catalyst the differentiating factor of which lies in the quantity of the material used in the experimental procedure. For instance, a catalyst is commonly used in less than stoichiometric amounts, a reagent is roughly used in stoichiometric amounts and solvents are used in amounts far higher than stoichiometric.

Table 4: Material category classification by definition

Material Category	**Definition**	**Reference**
Starting Material	A commodity material that contributes to the final API structure. It is commercially available from major reputable chemical companies at a cost of less than $100/mol of the greatest quantity offered and excludes bulk or custom-made quotations	(Roschangar *et al.*, 2018)
Advanced Starting Material	Advanced Starting Material is a non-commodity material that is significantly more complex than commonly available materials. These materials do not qualify as starting materials. Their synthesis routes and respective intrinsic E-factors are calculated and included in the overall synthesis greenness assessment.	(Roschangar *et al.*, 2018)
Reagent	A chemical substance that is added to a reaction in roughly stoichiometric proportions. They are used to initiate or enhance a reaction and may or may not be consumed within the reaction.	(Clark and Ekins, 2015)
Solvent	An ancillary chemical substance that dissolves another substance (solute) resulting in a homogeneous solution. Forms the major component of a solution. Solvents may also extract substances without chemically changing the chemical materials extracted. Used in amounts far higher than stoichiometric.	(Anastas and Zimmerman, 2018)

Catalyst	A substance, used in small amounts (much less than stoichiometric) that increases the rate of a chemical reaction without being consumed in the process.	(Prather, 2004)
Bio-catalyst	A catalyst of biological origin that is used in parts (isolated enzymes) or whole (microbial cells) to perform a chemical transformation.	(Prather, 2004)
Filter	A device or medium used to remove unwanted particles from a liquid or gas fluid.	(Augustyn *et.al.*, 2019)
Limiting reactant	The reactant that will be fully consumed first in a chemical reaction after which the reaction will cease to progress.	(Clayden, Greeves and Warren, 2012)
Molar Equivalence	The amount of a substance that reacts with one mole of the limiting reacting substance. It is calculated per reagent by dividing the number of moles of the reagent by the moles of the limiting reactant.	(Glossary of Organic Chemistry, 2018)

Experimental procedures that report solution mixtures were separated into their respective solute (reagent) and solvent components unless commercially available at the experimentally specified solution concentration. Additionally, H_2 gas passing over catalysts was classified as a reagent. Chemical materials reported more than once per synthesis step were added and placed under one chemical name and category.

Further, catalysts and bio-catalysts were excluded from the E-factor owing to the nature of these compound being regenerated and not forming part of the final API structure. Additionally, the *enantiomeric excess* percentage (*ee* %) reported considers the percentage composition of the desired and undesired geometry of the target molecule. The undesired enantiomer of the molecule was considered as an impurity which contributed to the E-factor.

The key formulas used to balance material masses between synthesis steps and scale up material quantities were:

A. Chemical yield: Defined as the amount of product produced when the limiting reagent (LR) has been fully consumed (Clayden, Greeves and Warren, 2012), mathematically represented as:

$$Yield = \frac{output\ mols\ product}{input\ mols\ LR} \times 100 \qquad (E3)$$

B. Molar Equivalence: Defined as the amount of one chemical substance that reacts with one mole of another chemical substance, commonly the LR, in a stoichiometric equation (Glossary of Organic Chemistry, 2018), mathematically represented as:

$$Molar\ Equivalent = \frac{input\ mols\ of\ material}{input\ mols\ LR} \qquad (E4)$$

The total materials costs of each synthetic pathway were determined by finding the cost of each chemical material and multiplying it by its required quantity. The price of each material was obtained from reputable commercial suppliers whereby the largest bulk quantity available and associated cost was selected. Equations E3 and E4 mathematically describe the costing method of each chemical material and entire synthetic pathway.

$$Material\ cost = \Sigma\ (v * cost\ per\ mole) \qquad (E5)$$

Where the cost per mole for each chemical material was calculated as illustrated in Appendix L and v is the stoichiometric factor or molar quantity of the chemical material used (as determined by the experimental procedure).

$$Total\ synthesis\ cost = \Sigma\ (material\ cost) \qquad (E6)$$

The total cost of the synthetic pathway was then divided by 10 to take into account the high prices of laboratory chemicals, making the cost more realistic (Sheldon, 2018a). This formed the basic economic evaluation of the synthetic pathways.

All material quantities and calculations were rounded off to two decimal places and three decimal places for smaller values. A detailed procedure describing the formation of the Excel spreadsheets is described in Appendix F.1.

4.2 Green Scorecards - iGAL™

iGAL™ determines the most mass-efficient synthetic pathway to an API target compound and quantifies its process greenness relative to industrial averages (RPG). The key process performance indicators (KPPI) used in determining iGAL™ are process complexity (CP), number of synthesis steps, complete E-Factor (cE-factor), salt free molecular weight (FMW) of the target compound and a consensus of starting material (commercially available raw materials at $\leq$ \$100/mol) (Roschangar, Sheldon and Senanayake, 2015). As described by Roschangar et al., (2018) the importance of determining the CP of a synthetic pathway is attributed to the iGAL™ algorithm showing trends in increased greenness performance with reduced CP. The original GAL™ defined the CP proxy as the summation of construction steps within a synthetic pathway, the details of differentiating between concession and construction steps represented in Appendix E.1 (Roschangar, Sheldon and Senanayake, 2015). The KPPI also facilitate greenness comparison amongst different target molecules (Roschangar et al., 2018).

The manual algorithm of iGAL™ as described by Roschangar et al., (2018) consists of the following steps:

1. Determine the FMW, cE-factor, CP and number of synthesis steps using the $\leq$ \$100/mol starting material rule,
2. Calculate the iGAL™ ($E7$, Section 4.2),
3. Calculate the RPG ($E8$, Section 4.2),
4. Use RPG matrix (Table 15 Section 4.2) to obtain rating,
5. Determine innovation impact (i.e., waste reduction and overall process improvements).

Moreover, Roschangar et al., (2018) developed a web based calculator known as the Green Chemistry Innovation Scorecard (Green Scorecard) to graphically represent and communicate the iGAL™ metric and effectively pinpoint the impacts of green chemistry in process design. It compares and communicates the KPPI of chemical pathways at three manufacturing stages i.e., early development (before clinical testing), late development and commercial stage within four quadrants corresponding to project status, waste reduction, innovation impact and performance versus industry benchmark. The scorecard effectively encourages the use of green chemistry in reducing process waste and thereby cost (Koenig and O'Brien, 2019). An example of the Green Scorecard™ interface with annotated adjustments made for the bis-THF alcohol assessment may be found in Appendix H.1. The Green Scorecard is freely available from the Innovation Quality Green Chemistry (IQGC) website https://www.acs.org/content/acs/en/greenchemistry/research-innovation/tools-for-green-chemistry/green-chemistry-innovation-scorecard-calculator.html

It is important to consider that iGAL™ and the Green Scorecard however only consider the mass-efficiency of synthetic procedures and do not account for process energy, safety and environmental impact categories which are generally considered in other early stage green assessment tools such as Green Motion™. The iGAL™ methodology may thus be considered as a complementary metric to more inclusive assessment tools (Roschangar, Colberg, Dunn, Gallou, Hayler, Koenig, Kopach, Leahy, Mergelsberg, Tucker, Sheldon and Senanayake, 2017).

4.3 Solvents

Over the past quarter decade, much progress has been made in finding greener solvent alternatives with 388 papers being published on the global improvement of solvent usage within pharmaceutical industries (Ashcroft, Dunn, Hayler and Wells, 2015). This is driven by environmental and societal needs along with the growing trend in replacing petrochemical with renewable biomass feedstocks. Further, bio-catalysts, aqueous bi-phasic catalysts and water are viable green alternatives to commonly used organic solvents. Despite this there remains reluctance to adopt greener alternatives owing to their

inconsistency in purity, cost and poorly supported greenness claims by subsequent studies (Sheldon, 2019).

Owing to the clear and pressing need for greening organic solvents, pharmaceutical companies including GSK, Pfizer and Sanofi etc., have developed in-house solvent guides that are in compliance with company policies and environmental regulations. The ACS GCI PR concluded that there is reasonable agreement amongst these publicly available solvent guides with slight discrepancies derived from differing company culture (ACS Green Chemistry Institute, 2014 as cited in Sheldon, 2019).

The first solvent guide was published by GSK in 1999 with the aim of reducing the large use of chlorinated solvents by 50% using greener non-chlorinated alternatives (A.D.Curzons, D.J.C.Constable and V.L.Cunningham, 1999 and Richard K. Henderson *et al.*, 2011). The popularity of chlorinated solvents was attributed to their high density and ease of separation from water (Alfonsi, Colberg, Dunn, Fevig, Jennings, Johnson, Kleine, Knight, Nagy, Perry and Stefaniakc, 2008). In 2003 the GSK solvent guide incorporated the LCA assessment as a result of numerous publications reporting their high impact contribution in API synthesis (Curzons *et al.*, 2001 and Jimenez-Gonzalez *et al.*, 2004). The guide was further expanded to include 154 solvent materials in 2016 (Alder, Hayler, Henderson, Redman, Shukla, Shuster and Sneddon, 2016). Throughout the progression of GSK solvent guides the overall approach in measuring the relative greenness of solvents remained relative to four sustainability categories namely: waste disposal, environmental impact, health and safety (EHS). For each category, which comprised of subcategories (Equations E9 – E12), solvents were assigned a score between 1 and 10.

Equations showing the four sustainability categories (LHS) and sub-categories (RHS) used in determining solvent scores, the details of which are discussed in Appendix G.1 (Alder, Hayler, Henderson, Redman, Shukla, Shuster and Sneddon, 2016):

$$\text{Waste} = \sqrt[4]{\text{Incineration} \times \text{Recycling} \times \text{Biotreatment} \times \text{VOC emission}} \qquad (E9)$$

$$\text{Environmental Impact} = \sqrt{\text{Air impact} \times \text{Aqueous impact}} \qquad (E10)$$

$$\text{Health} = \sqrt{\text{Exposure potential} \times \text{Health hazard}} \qquad (E11)$$

$$\text{Safety} = \sqrt{\text{Flammability and explosion potential} \times \text{Reactivity and stability}} \qquad (E12)$$

Depending on the individual category scores calculated; a colour using the traffic light notation was assigned to the solvent: red (score < 3.5), yellow ($3.5 \leq$ score < 7.5) or green (score ≥ 7.5) the meanings of which are illustrated in Table 5.

Table 5: Solvent colour ranking and its meaning

Ranking
Recommended
Recommended or Problematic (i.e., few issues)
Problematic (i.e., some issues)
Problematic or Hazardous (i.e., major issues)
Hazardous (i.e., should be banned)
Highly hazardous (i.e., should be banned)

After the individual scores were calculated a composite score described as the geometric mean of the four categories (waste, environmental impact, health and safety) was determined as illustrated by mathematical Equation E13.

$$\text{Composite} = \sqrt[4]{\text{Waste} \times \text{Environment Impacts} \times \text{Health} \times \text{Safety}} \qquad (E13)$$

Alder *et al.*, (2016) reported that solvent properties boiling point (bp) and vapour pressure (P_{vap}) feed into multiple assessment criteria, with high bp and low P_{vap} returning more favourable scores. Solvents P_{vap} however had the greatest influence in the composite score, forming part of five sub-categories namely, biotreatment, VOC emissions, air impact, exposure potential, flammability and explosion.

GSK solvent guides are award winning wed-based tools (Alder, Hayler, Henderson, Redman, Shukla, Shuster and Sneddon, 2016). These guides are freely available to scientists and engineers to find less hazardous and environmentally impactful materials in manufacturing API's. Pharmaceutical companies Pfizer and Sanofi have adopted similar solvent scoring systems. Other scoring methods such as the Green Motion™ tool score solvents based on a pictogram and health statements. Further, Henderson *et al.*, (2011) reported that quantitative calculations and comparison of solvent performance is unnecessary when visual aid solvent guides are available.

As mentioned in the literature review, solvents are responsible for a substantial amount of waste in synthesizing API's. Owing to this the relative greenness of the bis-THF alcohol synthesis routes assessed largely depends on the amount and nature of solvents used.

Solvent Intensity (SI) is a green metric that quantifies the environmental mass impact of solvents used in a chemical process. It is mathematically represented as (Curzons, Constable, Mortimera and Cunningham, 2001):

$$\frac{\Sigma m(Solvents)}{m(Product)} \qquad (E14)$$

Where the mass of product is the mass of the desired target compound. The optimum and hence most favorable value is zero, implying a solvent free synthesis with no environmental impact.

Assessing the nature of solvents is more complex and highly dependent on the evaluation system selected. The GSK solvent sustainability guide was selected to assess the nature of the solvents used in bis-THF alcohol synthetic pathways A, B and C. Appendix G.2, Figure G.2.1 illustrates a variety of commonly used organic solvents in the pharmaceutical industry with their respective GSK category scores and colour profiles. Figure 9 illustrates a more practical GSK solvent guide that categorizes solvents into their respective solvent classes (esters, aromatics, ketones etc.) for ease in comparing and identifying greener alternatives of structurally similar solvents. This guide further shows that ester, alcohol and carbonate solvent classes are generally greener than other solvent classes such as hydrocarbons, ethers and chlorinated hydrocarbons that are classified as problematic or hazardous based on toxicity and/or flammability issues.

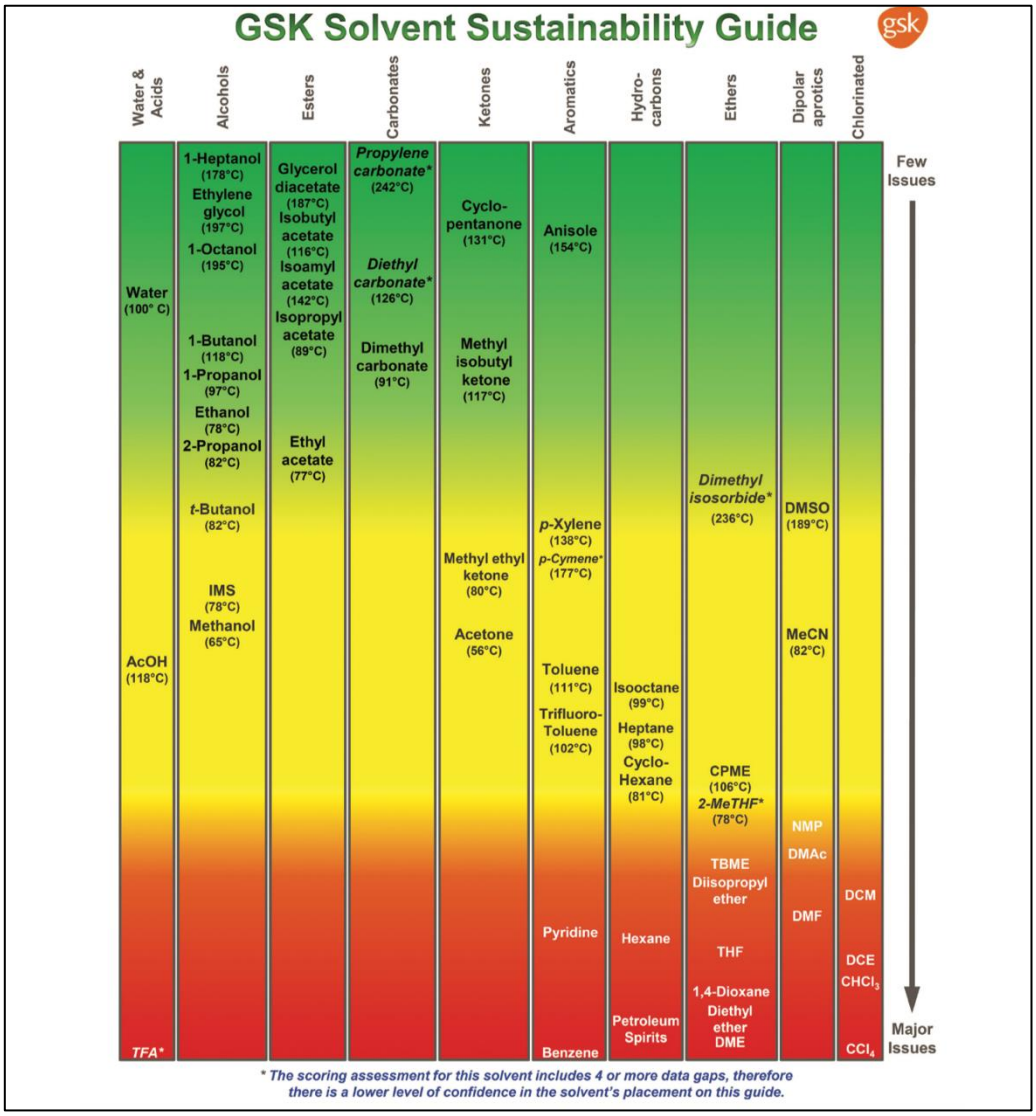


Figure 9: GSK solvent selection guide classified by chemical class (Alder et al., 2016)

4.4 Green Motion™

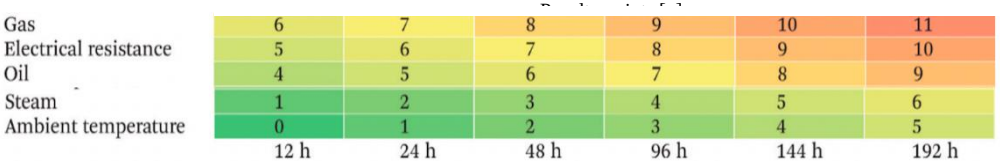
The Green Motion™ tool was formulated by reducing the twelve principles of green chemistry to seven fundamental concepts which are assessed to give a score, of between 0 and 100. The closer to 100 the rating the more sustainable the process and lower the environmental impact, promoting the development of less impactful processes and safer products.

The seven fundamental concepts include: starting material, solvent selection, hazard and toxicity of reagents, reaction efficiency, process efficiency, hazard and toxicity of final product and waste generation (MANE Flavours and Fragrances, 2018). To effectively assess each concept, a series of criteria were selected and translated into a questionnaire. The questions required simple answers which included: yes/no, pictograms, a numerical value or a selection from multiple choice options. Penalty points were deducted for undesired process operations selected (Phan, Gallardo and Mane, 2015). Table 6 describes the seven fundamental concepts with their respective assessment criteria, units and penalty points (where provided) as adapted from Phan, Gallardo and Mane, (2015) with additional details of each concept described in paragraphs thereafter. Mane refers to starting material as raw material.

After the Green Motion™ assessment has been completed, an output of concentric circles graphically representing the seven concepts, each with a score out of 100. The final, single cumulative environmental impact or Green Motion™ score was illustrated at the center of the concentric circles. This score integrates all seven fundamental concepts and hence all twelve principles of green chemistry. This implies that the health, safety, environmental, energy and waste impacts associated with the synthetic pathway were considered.

The input data required in performing the Green Motion™ assessment to the bis-THF alcohol was obtained from Pub Chem, Sigma Aldrich, E – factors and green metric values calculated from respective excel spreadsheets compiled from literature experimental procedures route A (Pandey et al., 2001; Janssen et al., 2011; Szczepankiewicz and Heathcock, 1997 and Hayashi *et al.*, 2016), route B (Sevenich, Liu, Arduengo III, Gupton and Opatz, 2017) and route C (Moore, Stringham, Teager and Yue, 2017)). Further, the health and safety measures were obtained from pictograms, collected and illustrated in answering the series of Green Motion™ questions in Appendix I.2.

Table 6: Green Motion™ seven fundamental concepts, criteria and units (Phan, Gallardo and Mane, 2015).

Number	*Concept*	Criteria	**Unit** [penalty points]
1	*Raw material*	Origin of material Natural process	**Category:** Synthetic [10], Hemi-synthesis [5], Natural [0] **Yes/No**
2	*Solvents*	Solvent class	**Category:** CMR [10], Petrochemical [5], Supercritical fluid [2], Renewable [2], Water [2], No solvent [0]
3	*Reagent/ solvent hazard & toxicity*	GHS pictogram	**Pictogram:** 
4	*Reaction*	Yield No. synthesis steps No. solvents Carbon economy No. protection/deprotection steps Overall process time	**Percentage value** **Numerical value** **Numerical value** **Percentage value** **Numerical value** **Numerical value (hours)**
5	*Process*	Heating Cooling Reaction under pressure Distillation Other process	**Category:**  **Yes/No** **Category:** Distillation under vacuum, Distillation at atmospheric pressure, No distillation [0] **Category:** Hazardous processes (nitrification, chlorination etc.), Energy-intensive process (liquid-liquid extraction, chromatography and so forth), None [0]
6	*Product hazard & toxicity*	GHS pictogram	**Pictogram:** 
7	*Waste*	E-factor	**Numerical value (kg.kg^{-1})**

Concept 1: Raw materials

Considers the origin of the starting material and the naturalness of the process. Natural starting materials are those obtained from vegetable, animal or microbiological origin. Natural process steps are those that do not change the nature of the chemical compound during the process operation such as, extraction and distillation, or involve only natural materials or catalysts, e.g., a microbiological transformation.

Concept 2: Solvent selection

Classified into 5 categories based on health, safety and environmental impacts. The highest penalty points are assigned to the carcinogenic, mutagenic and reprotoxic (CMR) solvents, and no penalty points to processes free from solvents.

Concept 3 and 6: Hazard and toxicity of solvents, reagents and products

The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) pictograms were used to assess the safety as well as provide warnings for each chemical material used. Each pictogram was assigned penalty points as guided by INERIS classification of hazard and toxicity. The greater the hazard the higher the penalty points (INERIS, 2010 as cited in Phan, Gallardo and Mane, 2015).

Concept 4: Reaction efficiency

Considers the possible criteria in which the reaction may be optimized. These are inclusive of: number of synthesis steps, reaction yield, catalysis, reaction selectivity (using protection and deprotection steps) as well as AE and CE.

Concept 5: Process efficiency

Focuses on the most energy consuming and conserving operations in a process including: heating, cooling, distillation and other pressure and energy intensive operations (including, but not limited to, crystallization and chlorination). These energy factors are not specifically quantified but are assigned penalty points depending on how energy intensive the method is and its operating time. For example, safer and more energy efficient steam heating was assigned fewer penalty points than gas heating over the same operating time. Zero penalty points are assigned to processes operating at ambient conditions under 12 hours.

Concept 7: Waste reduction

The traditional E-factor metric was the selected indicator for process waste generation. The E-factor metric was preferred over the PMI metric as it is additive and fits the Green Motion™ scoring method of zero penalty points being assigned to zero waste generation. Both the E-factor and the Green Motion™ tool are considered “gate-to-gate” assessments.

For the purpose of this assessment only concepts 3,4,5,6 and 7 were relevant in assessing the bis-THF alcohol synthetic methods (as described in detail in results and discussion Section 4.4). The GSK solvent guide was used in place of concept 2. Concept 1 is not really relevant in pharmaceuticals manufacture where only price and health and safety requirements are important.

4.5 Radial Polygon

The performance criteria selected in constructing the radial polygon were a variety of green mass metrics, green chemistry tool scores and process economics. Each octagonal vertex represents a performance criterion across all considered synthesis routes inclusive of cost, overall yield, E-factor, SI, wastewater intensity (WWI), purity, AE and Green Motion^{TM}.

It should be noted that the optimum value for cost, E-factor, SI and WWI is 0 whereas purity, overall yield, AE and Green Motion^{TM} have an optimum of 100 in their respective units. Owing to this, a ranking system (Appendix J) was constructed to score each performance criterion independently from best to worst and then join the performance of each criterion to form an irregular polygon. The area of the irregular polygon represents the wholistic performance of the synthetic pathway, considering all criteria assessed. The outermost octagon represents best performance (i.e., largest area) whereas the innermost octagon represents worst performance (i.e., smallest area). Thus, the less green the synthetic pathway, the greater the deformation of the octagon towards its centre. This aids in rapid identification of each synthesis route's strengths and weaknesses relative to each other. Hence the synthetic pathway with the greatest area was selected as the most eco-efficient route to the bis-THF alcohol. This provided a reasonable and succinct method for comparing the synthetic pathways.

5 Results and Discussion

5.1 Chemical Schemes and E-factor (Excel Spreadsheets).

5.1.1 General bis-THF alcohol routes discussion

The experimental procedures for route A, B and C were obtained from literature sources Hayashi et al. (2016); Sevenich *et al.*, (2017) and Moore *et al.*, (2017) respectively. The synthetic procedure to the advanced starting material (ASM) of route B was obtained from Sevenich *et al.*, (2017). The ASM for route A was created using a combination of literature sources including Janssen *et al.*, (2011); Szczepankiewicz and Heathcock, (1997); Tojo and Fernandez, (2007) and Pandey *et al.*, (2001).

Excel spreadsheets A, B and C were used as the basis for all green assessments and tools conducted in this dissertation. In assessing the three selected synthetic pathways to the bis-THF alcohol some terms, notation and drawings were created to simplify and communicate findings more eloquently. These terms and drawing formats are described and illustrated below:

Sub-synthesis: The synthetic pathway to ASM starting from commercially available starting materials found at $<$ 100$/mol from a reliable chemical vendor catalogue. The notation for these pathways is denoted by the subscript 'subordinate' e.g., route $A_{subordinate}$. The definition of ASM is imperative in finding a common starting point in assessing all synthetic pathways.

Main synthesis: The synthetic pathway as obtained from the respectively referenced literature experimental procedure. The starting materials described in these synthetic pathways were either ASM's or commercially available. The main synthetic pathways were either processed in a step-by-step or one-pot procedure, the details of which are described below:

- *Step-by-step procedure:* Defined as successive, individual synthesis steps with isolation and sometimes purification of intermediates that subsequently lead to the target molecule. This procedure may be described as a 'stop-and-go' method commonly performed to ensure each reaction step is compatible and operational under optimized reaction conditions (Hudlicky and Reed, 2007). The notation for this procedure was denoted by subscript 'step-by-step' e.g., route $B_{step\text{-}by\text{-}step}$.
- *One-pot procedure:* Defined as multi-step reactions, sequentially carried out in a single reaction vessel without intermediate isolation and purification steps (Wenbin, Zeng and Song, 2018). One-pot procedures are commonly devised after the step-by-step procedures have been analyzed and optimized. Such procedures are denoted with the subscript 'one-pot' e.g., route $B_{one\text{-}pot}$.

Entire synthesis: This is the complete synthetic pathway to the target compound (i.e., the combination of sub and main synthesis routes). The notation for these pathways are denoted by subscript 'entire' in conjunction with the main synthesis process e.g., route $B_{entire\ one\text{-}pot}$.

Intrinsic E – factor: The E-factor value associated with the sub-synthesis to an ASM.

Each synthetic pathway is visually represented by a schematic drawing the general format of which is illustrated in Figure 10. Organic solvents and reagents are represented by their respective abbreviated chemical names (e.g., isopropyl acetate = iPrOAc) or condensed chemical formulae (e.g., methanol = CH_3OH). If more than four solvents or reagents are used per step then only the largest quantities are illustrated in the synthetic pathway drawing. If temperature and pressure variations were described per step then one temperature and pressure range (lowest to highest) was illustrated for that step. Further, Figure 10 illustrates all the possible details that could be present in the schematic diagram, some of which were not available for all synthetic pathways assessed.

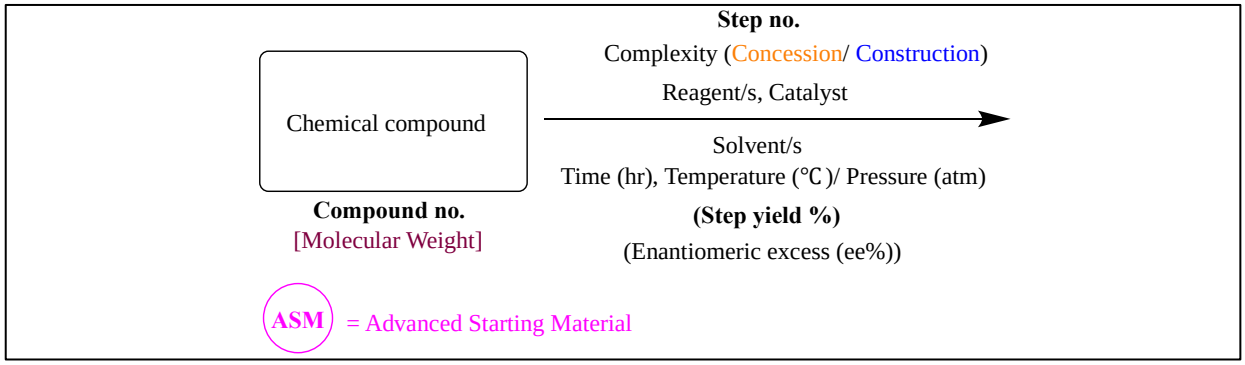
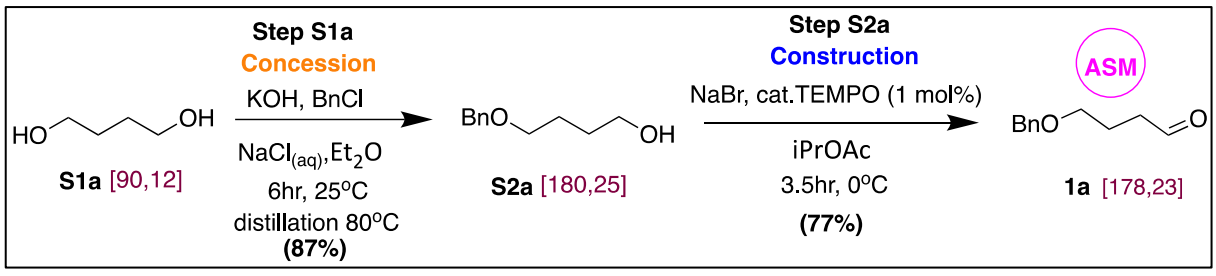


Figure 10: Legend for schematic representations of synthesis routes

The schematic representations and experimental descriptions for each synthetic pathway assessed are illustrated and discussed below. The molecular weights are shown in square brackets beneath the compounds.

Several characteristics, merits and limitations of each synthetic pathway to the bis-THF alcohol are described below. The E-factor including its variations was the metric selected to measure the material efficiencies for the production of bis-THF alcohol.

5.1.2 Route A Sub-synthesis



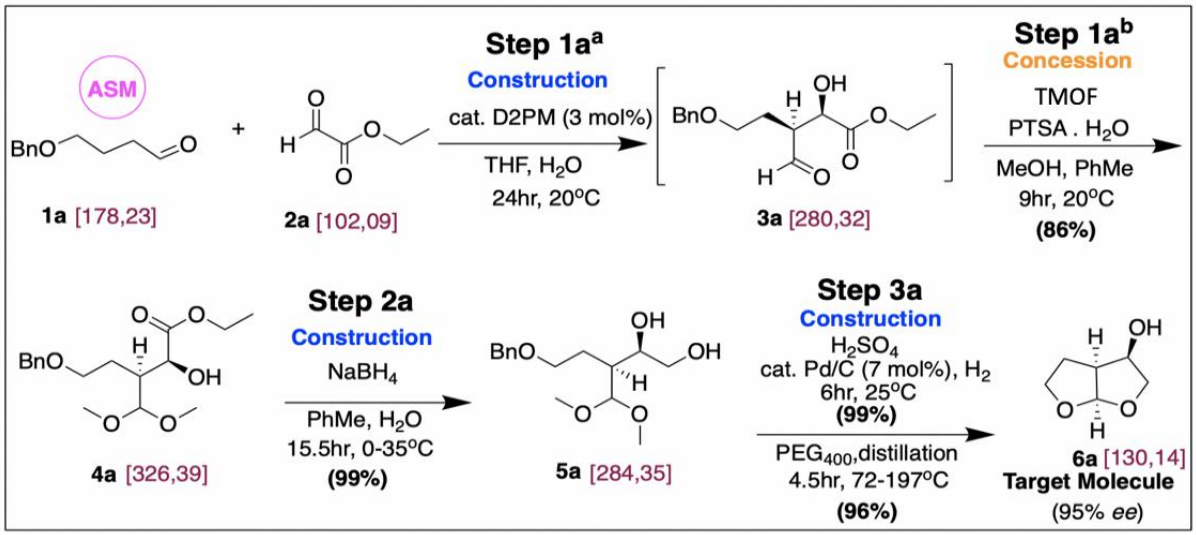
Scheme 1: Schematic representation of route $A_{subordinate}$ to ASM benzyloxy-butanal (1a) from butane-1,4- diol (S1a) and benzyl-chloride (Janssen et al., (2011); Szczepankiewicz and Heathcock, (1997); Tojo and Fernandez, (2007) and Pandey et al., (2001))

Scheme 1 illustrates the synthetic pathway to route A custom ASM benzyloxy-butanal (1a). The key synthesis step was the bleach oxidation of 4-benzyloxy-1-butanol to 4-benzyloxy-butanal (1a) using N-oxy catalyst TEMPO and iPrOAc as a green solvent. The synthetic pathway to ASM 1a was devised using a combination of literature sources including: Janssen *et al.*, (2011); Szczepankiewicz and Heathcock, (1997); Tojo and Fernandez, (2007) and Pandey *et al.*, (2001). The synthesis requires two synthesis steps starting from commercially available raw materials butane-1,4-diol (1.51$/mol), potassium hydroxide (2.14$/mol), sodium chloride (0.62$/mol) and benzyl chloride (5.03$/mol) that contains the benzyl (Bn) protection group. The Bn protection group is commonly perceived to be a more challenging protection group to add and remove from molecules than the Cbz protecting group used in route $B_{subordinate}$ procedure (Scheme 3).

Additionally, the catalytic reagent TEMPO was used at a low catalyst loading of 1 mol% and was found to be ineffective in lower catalytic amounts when experimentally tested (Janssen, Castellana, Jackman, Dunnb and Sheldon, 2011). The catalyst was not used in stoichiometric quantities thereby obeying the 9th principle of green chemistry. Additionally, TEMPO requires low operating temperatures to prevent inactivation of the catalyst and unwanted side reactions which adheres to the 6th principle of green chemistry. Further, NaBr was used as the co-catalyst in 10 mol% along with 1.05 molar equivalents of NaOCl reagent (Janssen, Castellana, Jackman, Dunnb and Sheldon, 2011). A measure of 5 mol% $NaHCO_3$ was used to buffer the solution to pH 9.5 to avoid unwanted hydrolysis side reaction in the presence of the isopropyl acetate (Tojo and Fernandez, 2007). This method requires a buffered biphasic system (isopropyl acetate: water in a 1:4.5 mass ratio) (Janssen *et al.*, 2011 and Tojo and Fernandez, (2007)). Liquid-liquid extraction using water and iPrOAc was then performed to separate the organic layers. The operating temperature and pressure of this separation were at ambient conditions. It is important to consider that di-ethyl ether (Et_2O) is a highly hazardous inflammable solvent which may not be used at an industrial scale, however since it is used as an extraction solvent it may be easily replaced by iPrOAc to use directly in the second synthesis step.

A condensed experimental procedure of the synthetic scheme illustrated in Scheme 1, includes: StepS1a the preparation of 4-benzyloxy-1-butanol (S2a) from commonly available starting material butane-1,4-diol (S1a) in 87% yield as reported by Pandey *et al.*, (2001) along with the fractional distillation at 80℃ to obtain purified S2a compound; subsequently stepS2a used the general bleach oxidation procedure to convert the alcohol to the aldehyde as reported by Janssen *et al.*, (2011) in 77% yield as reported by Szczepankiewicz and Heathcock, (1997). The overall synthesis yield of route $A_{subordinate}$ was calculated at 67% with an intrinsic E-factor of 3.10, cE-Factor of 32.40 and sE-Factor of 1.49 in producing 1 kg ASM benzyloxy-butanal (Excel spreadsheet A) with a synthesis complexity of 1. The breakdown of the material masses required per synthesis step for route $A_{subordinate}$ in producing 1 kg ASM benzyloxy-butanal (1a) is represented in Appendix F.3.

5.1.3 Route A Main-synthesis



Scheme 2: Schematic representation of route $A_{step\text{-}by\text{-}step}$ synthesis of bis-THF alcohol (6a) from ASM 4-benzyloxybutanal (1a) and ethyl glyoxylate (2a) (Hayashi et al.,2016). Superscripts a and b in the scheme refer to the internal intermediate

Scheme 2 illustrates the main route $A_{step\text{-}by\text{-}step}$ synthetic pathway to bis-THF alcohol as described by Hayashi *et al.*, (2016). The key synthesis step involves an *(S)* - diphenylprolinol (D2PM) catalyzed highly enantio - and diastereoselective cross aldol reaction of ethyl glyoxylate (2a) with a Bn-protected aldehyde (1a). This synthetic pathway is an improved version of a first-generation synthesis route (i.e., avoiding the use of expensive benzyloxyacetaldehyde and redox reaction to obtain the desired bis-THF alcohol diastereomer) as reported by Ikemoto and Watanabe, (2006). The starting materials selected were ethyl glyoxylate (2a) and Bn-protected aldehyde (1a) based on the report by Urushima *et al.*, (2010) of analogous highly stereospecific aldol reactions using *(S)* - diphenylprolinol (D2PM) catalyst. Starting material 2a was commercially available in its polymeric form at a relatively low cost (10.21 $/mol) and could be directly used without pyrolysis. This made the synthesis highly practical, stereoselective and environmentally friendly with reduced energy demands.

Further, environmentally benign D2PM organocatalyst was used in small quantity of 3 mol%, with retained catalyst activity. The low catalyst loading, and good reproducibility ensured that the synthesis was practical and cost effective. At lower D2MP catalyst concentrations the reaction did not run to completion. Additionally, the use of green catalytic hydrogenation with Pd/C is stoichiometric reduction with low molecular weight $NaBH_4$ thus having low Atom Economy (AE), producing little waste adhering to the green chemistry principle 2 and 9.

The PEG_{400} solvent was used as a ‘chaser solvent’ in the final distillation step of the route $A_{step\text{-}by\text{-}step}$ procedure to purify the bis-THF alcohol. A high-boiling point ‘chaser solvent’ avoids product losses in

the ‘dead volume’ of the distillation column (Kazakevich, 2007) by chasing out the desired product. Recently, PEG has gained much attention as an inexpensive and eco-friendly organic solvent (10 $/kg (Nt-itrade, 2019)) having excellent compatibility with a number of chemical transformations (Vafaeezadeh and Hashemi, 2015).

Additionally, the concentration of chemical materials and intermediates in the experimental procedure are reported as percentage assay. A percentage assay represents the quantitative measure of the analyte (i.e., the chemical entity being measured) with the remainder representing impure materials i.e., impure intermediates and end products (Braun, 2016), which are accounted for in the E-factor calculations.

A condensed description of the experimental procedure and results as demonstrated in Scheme 2 are as follows: Step $1a^{a+b}$ entailed the key synthesis step in preparation of ethyl (2*R*,3*S*)-2-hydroxy-3-dimethoxy-methyl-5-benzyloxypentanoate (4a) from commonly available starting material ethyl glyoxylate (2a) and ASM benzyloxy-butanal (1a) in 86% yield. Further, step 2a entailed stoichiometric reduction of an ester functionality to the corresponding alcohol using sodium borohydride to form (2*R*,3*S*)-3-Dimethoxymethyl-5-benzyloxy-1,2-pentanediol (5a) in 99% yield. The final synthesis step 3a entailed the removal of Bn protection group by Pd/C catalysed hydrogenolysis and purification of the resulting bis-THF alcohol in 96% yield and 95% *ee*. The overall synthesis yield for route $A_{step\text{-}by\text{-}step}$ was 81% with an E-factor calculated at 7.27, cE-Factor of 41.07 and sE-Factor of 9.29 (Excel spreadsheet A) and a synthesis complexity of 3. The breakdown of the material masses required per synthesis step for route $A_{step\text{-}by\text{-}step}$ in producing 1 kg bis-THF alcohol (1a) is represented in Appendix F.3.

5.1.4 Contribution of intrinsic E-factor to main synthesis and cumulative route A

As previously represented, route A consist of ASM benzyloxy-butanal (1a) that was not commercially available thus requiring its own synthetic pathway to meet the ≤ $100/mol starting material definition (Roschangar, Sheldon and Senanayake, 2015). Combining the ASM synthesis with the main synthesis route indisputably led to an increase in synthesis steps, reaction complexity, chemical materials and total waste (E-factor) in synthesizing the bis-THF alcohol.

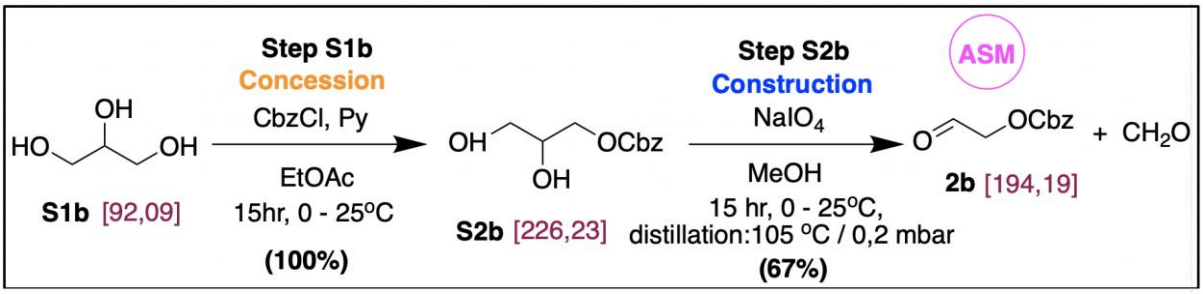
Table 7: Route $A_{subordinate}$ intrinsic E-Factor contribution of ASM 1a to route $A_{step\text{-}by\text{-}step}$ procedure in producing 1 kg bis-THF alcohol.

Simple E-factor	**Complete E-factor**	**E-factor**
Contribution to compound 1a sub-process(kg/kg)		
1.49	32.40	3.10
× *Quantity required to produce 1 kg of bis-THF alcohol (kg)*		
1.69		
Contribution to Route A step-by-step synthesis to bis-THF alcohol (kg/kg)		
2.52	54.76	5.24

Table 7 illustrates that 1.69 kg of ASM benzyloxy-butanal (1a) was required in formulating 1 kg bis-THF alcohol in route $A_{step\text{-}by\text{-}step}$ procedure, as predicted in Excel spreadsheet A. This contributes to an added waste of 5.24 kg per 1 kg bis-THF alcohol. The waste (E- Factor) of route $A_{step\text{-}by\text{-}step}$ thus increased from 9.29 to 14.54. Thus, in disregarding the waste associated with route $A_{subordinate}$, 57% of the total E-factor for route $A_{entire\ step\text{-}by\text{-}step}$ procedure would have been neglected. Additionally, the cE-Factor increased from 41.07 to 95.82 and sE-Factor from 7.27 to 9.79.

Further in combining the ASM and the main synthesis route the cumulative complexity was 4, the overall yield was 54%, the E-factor was 14.54, and the total number of synthesis steps was 5. All of these details were required in the Green Scorecard assessment in Section 4.2. Additional details containing the collective mass and percentage compositions of chemical materials used in each of the route A scenarios are illustrated in Appendix F.3.

5.1.5 Route B Sub-synthesis

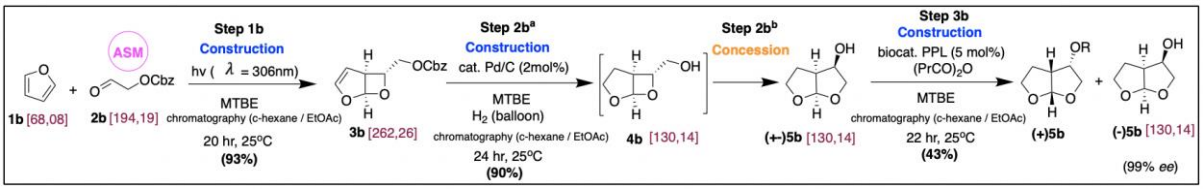


Scheme 3: Schematic representation of route $B_{subordinate}$ to ASM Cbz-aldehyde (2b) from glycerol (S1b) and benzyl chloroformate (Sevenich et al., 2017).

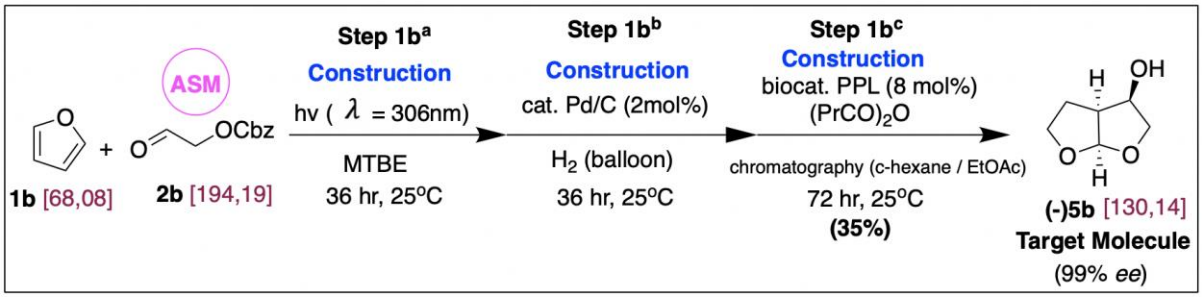
Scheme 3 illustrates the synthetic pathway to route B custom ASM carboxybenzyl (Cbz) - protected aldehyde (2b). The Cbz group was selected as the protection group as it is inexpensive (found as benzyl chloroformate at 88.54$/mol), easy to introduce and remove and it is inert to the hydrogenation reaction conditions. It was thus found to be more favourable than the Bn protecting group used in route $A_{subordinate}$. Sevenich *et al.*, (2017) reported the Cbz protection group to be feasible after being tested under optimized reaction conditions. Three alternative experimental procedures were reported. The first involved the use of toluene (PhMe) and ethylene glycol as starting materials, both of which may be obtained from renewable woody biomass. The second involved the use of glycerol and dichloromethane (DCM) and performed better than the first method on scale-up but posed higher toxicity and health risk (Sevenich *et al.*, 2017). The third method (Scheme 3) was selected as it did not involve the use of hazardous chlorinated solvents and afforded the highest purity after large scale distillation. The feasibility of the third procedure was demonstrated under optimized reaction conditions. It required two synthesis steps from commercially available starting materials glycerol (1.84$/mol) and pyridine (8.89$/mol).

As shown in Scheme 3, step S1b entailed the preparation of Cbz – protected glycerol (S3b) from glycerol (S1b) and benzyl chloroformate (S2b) in 100% yield and step S2b involved oxidative cleavage of the diol moiety by sodium periodate and distillation to afford the Cbz-protected hydroxyacetaldehyde in 67% yield. The overall yield of route $B_{subordinate}$ was calculated at 67% with an intrinsic E-factor calculated at 15.76 (Excel spreadsheet B) and a synthesis complexity of 1. The breakdown of the material masses required per synthesis step for route $B_{subordinate}$ in producing 1kg ASM Cbz-protected glycol aldehyde (2b) is represented in Appendix F.4.

5.1.6 Route B Main-synthesis



Scheme 4: Schematic representation of route $B_{step\text{-}by\text{-}step}$ procedure to bis-THF alcohol (±5b) from ASM Cbz-protected aldehyde (2b) and furan (1b) (Sevenich et al., 2017)



Scheme 5: Schematic representation of route $B_{one\text{-}pot}$ procedure to bis-THF alcohol ((-)5b) from ASM Cbz protected aldehyde (2b) and furan (1b) (Sevenich et al., 2017).

Scheme 4 and Scheme 5 illustrate the main route $B_{step\text{-}by\text{-}step}$ and route $B_{one\text{-}pot}$ to bis-THF alcohol, respectively, as reported by Sevenich *et al.*, (2017). Both pathways require the same chemical materials and key synthesis steps but are processed differently. The step-by-step experimental procedure was conducted to optimize reaction conditions and select the best performing materials (biocatalysts etc.) such that the one-pot procedure performed optimally. The disadvantage of route $B_{step\text{-}by\text{-}step}$ was the purification of chemical intermediates after each synthesis step which led to large amounts of solvent waste and an increased E-factor. The key synthesis step is the photocycloaddition between furan (1b) and Cbz-protected glycol aldehyde (2b), followed by the hydrogenation and enzymatic optical resolution. It was an improved version of a first-generation synthesis reported by Doan, (2003). Improvements included the use of inexpensive, renewable starting materials, avoiding the use of chlorinated solvents, and a reduction in the number of purification steps to increase the environmental sustainability of the process.

A key merit of both route B procedures was the use of inexpensive starting material furan ($8.90/mol) derived from renewable woody biomass, meeting the criteria for xylochemistry[7]. Additionally, solvents glycerol and PhMe, forming the carbon skeleton of the bis-THF alcohol, can also be obtained from woody biomass, more specifically from pine wood cellulose solids. Xylochemistry complies with the

[7] Xylochemistry makes use of wood as the raw material for a chemical synthesis. It focuses on developing economic and environmentally sustainable manufacturing methods from wood to commodity chemical materials and pharmaceuticals (Opatz and Arduengo, 2019) .

7th principle of green chemistry and provides a CO_2 neutral alternative to the conventionally used fossil fuels, thus promoting a more sustainable manner in manufacturing the bis-THF alcohol (Opatz and Arduengo, 2019). A further merit is the use of three environmentally acceptable solvents (ethyl acetate (EtOAc), methyl tert-butyl ether (MTBE), cyclohexane (c-hexane)) that are easy to recovery and recycle.

The reaction was a [2+ 2] photo cycloaddition performed between furan and Cbz-protected aldehyde which took place over 36 hours in a quartz glassware aligned with an array of 16 UV Rayonet lamps ($\lambda_{max} = 306$nm each at 7.2W), and a cooling fan. It constitutes a key step in that it generates the relevant stereochemistry (all three stereogenic centres in the bis-THF moiety in the correct configuration (Appendix C.3)), by producing a racemate where only one of the two compounds has the correct absolute stereochemistry. Subsequent removal of the Cbz - protection group and enzymatic resolution of the racemic product afforded the desired bis-THF alcohol (Sevenich, Liu, Arduengo III, Gupton and Opatz, 2017).

Both route B procedures make use of hydrolytic biocatalyst Porcine Pancreatic Lipase (PPL), immobilized on polymeric beads to increase their stability, which operates at low temperatures and pressures, allowing for multiple enzyme re-use and simple product recovery (Homaei, Sariri, Vianello and Stevanato, 2013). Conversely, PPL has the demerit of being derived from animal origin (pig pancreas) which is not acceptable in API manufacture. A possible recommendation is that the PPL can be replaced by a microbial alternative such as *Pseudomonas cepacia* lipase (PcL). Further, commercially available PPL is a crude enzyme preparation containing other enzymes, e.g., chymotrypsin and cholesterol esterase (Wong and Whitesides, 1994) which can lead to lower selectivities. A further demerit of the route B procedures lies in the low overall yield which is a reflection of the late kinetic resolution at the last stage of the synthesis. According to Sheldon (1996), the 'golden rule' is to introduce the resolution step as early as possible within the synthesis to eliminate the waste carry through on all materials.

As shown in Scheme 4, route $B_{step\text{-}by\text{-}step}$ entails the photochemical reaction (step 1b) between the ASM 2b and 1b to give exo-2,7-dioxabicyclo[3.2.0]hept-3-en-6-ylmethyl benzyl carbonate (3b) in 93% yield. Subsequently catalytic hydrogenation at ambient temperatures and pressures (step 2b) leads to the formation of anti-hexahydrofuro[2,3-b]furan-3-ol ($\pm$5b) in 90% yield. The final synthesis step 3b entailed the lipase-catalysed kinetic resolution to afford the bis-THF alcohol in 43% yield at room temperature. The overall yield for route $B_{step\text{-}by\text{-}step}$ procedure was 36% with an E-factor calculated at 61.41, cE-Factor of 519.67 and sE-Factor of 10.49 (Excel spreadsheet B). Route $B_{one\text{-}pot}$ (Scheme 5) consists of the same experimental operations as route $B_{step\text{-}by\text{-}step}$ but they are performed sequentially in a single reaction vessel, affording an overall yield of 35% and an E-factor of 22.49, cE-Factor of 102.15 and sE-Factor of 13.64 (Excel spreadsheet B) and a complexity of 3. The breakdown of the material

masses required per synthesis step for route $B_{step\text{-}by\text{-}step}$ and route $B_{one\text{-}pot}$ in producing 1 kg bis-THF alcohol are represented in Appendix F.4.

5.1.7 Contribution of intrinsic E-factor to main Route B synthesis

As previously represented, route B consist of ASM Cbz-aldehyde (2b) that was not commercially available thus requiring its own synthetic pathway to meet the ≤ $100/mol starting material definition (Roschangar, Sheldon and Senanayake, 2015).

Table 8: Route $B_{subordinate}$ intrinsic E-Factor contribution of ASM 2b to route $B_{step\text{-}by\text{-}step}$ procedure in producing 1 bis-THF alcohol.

Simple E-factor	**Complete E-factor**	**E-factor**
Contribution to compound 2b sub-process (kg/kg)		
10.50	63.12	15.76
× *Quantity required to produce 1 kg of bis-THF alcohol (kg)*		
4.15		
*Contribution to Route B **step-by-step** synthesis to bis-THF alcohol (kg/kg)*		
43.54	261.72	65.36

Table 9: Route $B_{subordinate}$ intrinsic E-Factor contribution of ASM 2b to route $B_{one\text{-}pot}$ procedure in producing 1 kg bis-THF alcohol.

Simple E-factor	**Complete E-factor**	**E-factor**
Contribution to compound 2b sub-process (kg/kg)		
10.50	63.12	15.76
× *Quantity required to produce 1 kg of bis-THF alcohol (kg)*		
4.26		
*Contribution to Route B **one-pot** synthesis to bis-THF alcohol (kg/kg)*		
44.77	269.10	67.20

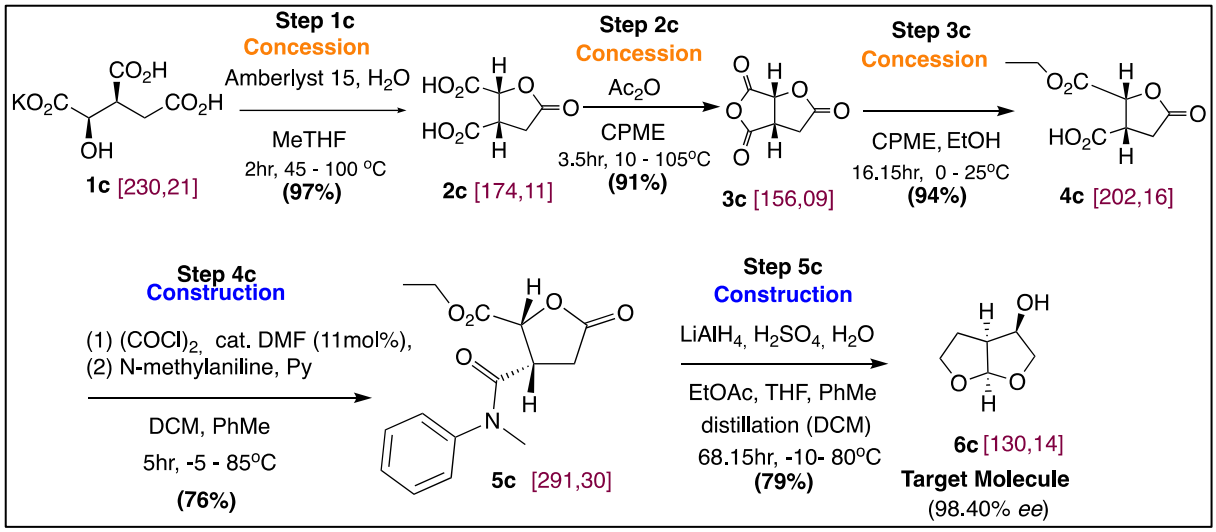
Table 8 and Table 9 show that 4.15 kg and 4.26 kg of ASM Cbz-protected glycol aldehyde (2b) was required in formulating 1 kg bis-THF alcohol in route $B_{step\text{-}by\text{-}step}$ and route $B_{one\text{-}pot}$ procedure respectively. This contributes to an added waste (E-factor) of 65.36 kg for route $B_{step\text{-}by\text{-}step}$ and 67.20 kg for route $B_{one\text{-}pot}$ procedure. The waste of route $B_{step\text{-}by\text{-}step}$ and route $B_{one\text{-}pot}$ thus increased from 61.41 to 126.77 and 22.49 to 89.69, respectively. Thus, in disregarding the waste of route $B_{subordinate}$, 52% and 75% of the total E-Factor for route $B_{entire\ step\text{-}by\text{-}step}$ and route $B_{entire\ one\text{-}pot}$ procedure would have been neglected, respectively. Additionally, for route $B_{entire\ step\text{-}by\text{-}step}$ the cE-Factor increased from 519.67 to

781.39 and sE-Factor increased from 10.49 to 54.03. Further for route $B_{entire\ one\text{-}pot}$ the cE-Factor increased from 102.15 to 371.25 and the sE-Factor increased from 13.64 to 58.41.

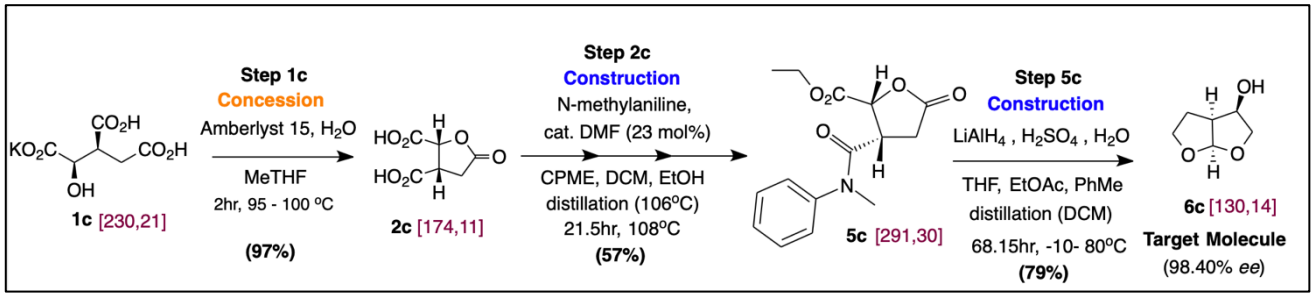
Moreover in combining the ASM and route $B_{step\text{-}by\text{-}step}$ procedure the cumulative complexity was 4, the overall yield was 24%, the E-factor was 126.77, and the total number of synthesis steps was 5. In combining the ASM and route $B_{one\text{-}pot}$ procedure the cumulative complexity was 4, the overall yield was 23%, the E-factor was 89.69, and the total number of synthesis steps was 2.

The main difference between the step-by-step and one-pot procedure is the reduction in the total number of synthesis steps from 5 to 2 and the large reduction in solvent quantity (c-hexane, EtOAc and MTBE). The one-pot procedure was further reported to operate under optimized reaction conditions, but the condensed synthetic pathway required an increase in quantities of ASM, reagents propionic anhydride and furan as well as PPL biocatalyst. Additional details containing the collective mass and percentage compositions of chemical materials used in each of the route B scenarios is illustrated in Appendix F.4.

5.1.8 Route C Main-synthesis



Scheme 6: Schematic representation of route $C_{step\text{-}by\text{-}step}$ procedure to bis-THF alcohol (6c) from potassium isocitrate (1c) (Moore et al., 2017).



Scheme 7: Schematic representation of route C $_{one\text{-}pot}$ procedure to bis-THF alcohol (6c) from potassium isocitrate (1c) (Moore et al., 2017).

Scheme 6 and Scheme 7 illustrate the main route $C_{step\text{-}by\text{-}step}$ and route $C_{one\text{-}pot}$ synthetic pathway to bis-THF alcohol respectively as reported by Moore *et al.*, (2017). The key step in route C main synthesis was the identification of the amide N-methyl aniline as the optimum substrate for the reduction to bis-THF alcohol. This synthetic pathway was an improved version of a first-generation synthesis reported by Heretsch et al, 2008. The key merit of route C lies in the use of a naturally occurring, enantiomerically pure starting material, monopotassium isocitrate (1c) that is not an ASM and available at a cost as low as $50/kg (Moore et al., 2017). This starting material was obtained from a high-yielding fermentation process fed by sunflower oil using the Jen41 strain of yeast *Yarrowia lipolytica* eliminating the need for a resolution or asymmetric synthesis step at later stages of the synthetic scheme. As reported by Sheldon (1996), introducing the resolution step as early as possible within the synthetic procedure as possible eliminates the carry through of 50% undesired enantiomer thus reducing material quantity and cost of the synthesis. Hence, route C eliminates the need for optical resolution and affords an optically pure bis-THF alcohol directly.

The demerits of both route C procedures are the use of a large variety of solvents, which may cause problems at product purification stages and large volumes of water were used during the neutralization using ion exchange resin. Further, the route generates toxic and hazardous N-methyl aniline as a by-product which goes against the fundamentals of green chemistry.

A condensed description of route $C_{step\text{-}by\text{-}step}$ experimental procedure and results as demonstrated in Scheme 6 are as follows: Step 1c entails the conversion of potassium isocitrate (1c) using ion exchange resin Amberlyst 15 to (2*R*,3*S*)-5-oxytetrahydrofuran-2,3-dicarboxylic acid (2c) in 97% yield. Subsequently, step 2c entailed the cyclic anhydride formation of (3a*S*,6a*R*)-dihydrofuro[3,4-b] furan-2,4,6(3H)-trione (3c) using 1.18 molar equivalents of Ac_2O in 91% yield. Step 3c required the suspension of compound 3c in solvent CPME and reaction within an anhydride-opening reaction to afford (2*R*,3*S*)-2-(ethoxycarbonyl)-5-oxotetrahydrofuran-3-carboxylic acid (4c) in 94% yield. Subsequently, step 4c performed an acyl chloride formation and the latter is reacted with N-methylaniline, carried out in DCM as a solvent and DMF as a catalyst, in the presence of Py to afford ethyl (2*R*,3*S*)-3-(methyl(phenyl)carbamoyl)-5-oxotetrahydrofuran-2-carboxylate (5c) in 76% yield. DMF is commonly used as a solvent (undesired) but in this synthesis it is used as a catalyst. The final synthesis step 5c entailed that compound 5c was reduced with $LiAlH_4$ in THF, treated with H_2SO_4, transferred to a continuous extractor and extracted with DCM after which DCM was evaporated and the crude oil was dissolved in EtOAc, filtered through silica gel and the EtOAc evaporated to afford the bis-THF alcohol (6c) in 79% yield. The overall yield for route $C_{step\text{-}by\text{-}step}$ procedure was 50% with an E-factor calculated at 124.85, cE-Factor of 1177.54 and sE-Factor of 70.90 (Excel spreadsheet C) and a complexity of 2. The breakdown of the material masses required per synthesis step for route $C_{step\text{-}by\text{-}step}$ and route $C_{one\text{-}pot}$ in producing 1 kg bis-THF alcohol are represented in Appendix F.5.

Similarly, route $C_{one\text{-}pot}$ procedure demonstrated in Scheme 7 has a similar experimental operation to route $C_{step\text{-}by\text{-}step}$ however a portion of the synthetic pathway was a one-pot procedure (i.e., from step 2c to step 5c). The difference between the step-by-step and one-pot procedure is that no oxalyl chloride is used in forming the amide (5c) and the total number of synthesis steps are reduced from 5 to 3. The one-pot procedure was thus incorporated to condense the synthetic pathway but required an increase in quantities of CPME and DCM solvents as well as DMF catalyst. The one-pot procedure was also not reported to perform under optimized reaction conditions. Thus, the overall synthesis yield for route $C_{one\text{-}pot}$ procedure was 44% with an E-factor calculated at 273.37, cE-Factor of 1626.35 and sE-Factor of 207.19 (Excel spreadsheet C) and a complexity of 2. The breakdown of the material masses required per synthesis step for and route $C_{one\text{-}pot}$ in producing 1 kg bis-THF alcohol are represented in Appendix F.5.

Thus, for route $C_{step\text{-}by\text{-}step}$ procedure the complexity was 2, the overall yield was 50%, the E-factor was 124.85, and the total number of synthesis steps was 5. For route $C_{one\text{-}pot}$ procedure the complexity was 2, the overall yield was 44%, the E-factor was 207.19, and the total number of synthesis steps was 3. Additional details containing the collective mass and percentage compositions of chemical materials used in each of the route C scenarios are illustrated in Appendix F.5.

5.1.9 Summary of all synthetic pathways material masses and E-Factor derivatives

Table 10 illustrates a summary of material masses required per category in synthesising 1 kg target compound for each respective synthetic pathway.

Table 10: Mass quantities of materials per category per synthesis route in producing 1 kg bis-THF alcohol and respective E-factor derivative values.

Synthesis Route							
	$\mathbf{A}_{\text{subordinate}}$	$\mathbf{A}_{\text{step-by-step}}$	$\mathbf{B}_{\text{subordinate}}$	$\mathbf{B}_{\text{step-by-step}}$	$\mathbf{B}_{\text{one-pot}}$	$\mathbf{C}_{\text{step-by-step}}$	$\mathbf{C}_{\text{one-pot}}$
Material Category Masses							
Starting Material (kg)	0.75	1.69	7.05	4.15	4.26	3.55	4.05
Solvent (kg)	16.14	20.22	52.62	509.17	88.51	539.47	661.83
Reagent (kg)	1.73	6.58	4.45	7.35	10.37	68.34	204.14
Water (kg)	14.77	13.58	0.00	0.00	0.00	567.17	757.33
E-Factor derivative values							
sE-Factor (kg/kg)	2.52	7.27	43.54	10.49	13.64	70.90	207.19
E-Factor (kg/kg)	5.24	9.29	65.36	61.41	22.49	124.84	273.37
cE-Factor (kg/kg)	54.76	41.07	261.72	519.67	102.15	1177.54	1626.35

Further, Table 11 represents a summary of the waste generation and synthesis details of each entire pathway (i.e., combining sub and main synthesis route) in producing 1 kg bis-THF alcohol. Thus these pathways are inclusive of ASM synthesis yields, E-Factors etc., as required in routes A and B. The detailed calculations in obtaining these values may be found in Appendix F and Excel spreadsheets A, B and C.

Table 11: E-Factor derivative values and synthesis details for entire route procedures of all synthetic pathways assessed.

	Simple E-factor (kg/kg)	**Complete E-factor (kg/kg)**	**E-factor (kg/kg)**	**Complexity** (Σ Construction steps)	**No. synthesis steps**	**Overall Yield (%)**
Route $A_{\text{entire step-by-step}}$	9.79	95.82	14.54	4	5	54
Route $B_{\text{entire step-by-step}}$	54.03	781.38	126.77	4	5	24
Route $B_{\text{entire one-pot}}$	58.40	371.25	89.69	4	2	23
Route $C_{\text{step-by-step}}$	70.90	1177.54	124.84	2	5	50
Route $C_{\text{one-pot}}$	207.19	1626.35	273.37	2	3	44

Further, the average E-factor of all routes A, B and C is 125.84 (> 95 *ee*% purity) which compares well with the ACS GCIPR E-factor average of 130. However, it is unacceptably high when one considers that the bis-THF alcohol is a sub-unit of an API with a molecular weight (MW) of 130.14 g/mol) and not a complete API structure with an average MW of 451 as in the ACS GCIPR study. On the other hand, the ACS GCIPR is concerned with scaled up processes.

5.1.10 E-factors and Cost

The total cost of each synthetic pathway was determined by finding the largest bulk quantity of the material available from a reputable commercial supplier (Sigma-Aldrich catalogue, February 2019, unless otherwise stated). The molar price of each chemical material was then calculated and multiplied by the molar quantity of the material as required in the experimental procedure (details described in Appendix L). The individual costing of materials was divided by 10 to take into account the high prices of laboratory chemicals as represented in Excel Spreadsheets A, B and C. Further, since the starting materials of the synthesis routes were identified using the <100$/mol rule defined by Roschangar *et al.*, (2018), the costing of all other chemical materials used were quoted using the same units ($/mol).

Table 12 illustrates the cost and respective cE-factors for each synthetic pathway used to make the same amount of product i.e., 1 kg bis-THF alcohol. The cE-factor was the selected waste metric as it accounts for the maximum possible waste associated with a synthesis. In other words, it considers all process materials required, including work-up solvents and water, with no material re-cycling. The former showed that route $A_{\text{entire step-by-step}}$ has a 92% cE-factor reduction and 60% cost improvement compared to route $C_{\text{step-by-step}}$ procedure. This suggests that a waste reduction of over 90% with a 60% reduction in process cost may be achieved in manufacturing the same amount of product.

Table 12: Cost and cE-factor values for the synthesis of 1 kg bis-THF alcohol

Synthesis route	Cost ($/kg)	cE -factor (kg/kg)
$A_{\text{entire step-by-step}}$	1285.68	95.82
$B_{\text{entire step-by-step}}$	1663.37	781.38
$B_{\text{entire one-pot}}$	739.19	371.25
$C_{\text{step-by-step}}$	3189.15	1177.54
$C_{\text{one-pot}}$	4998.88	1626.35

Further, from Table 12 it is evident that route $A_{\text{entire step-by-step}}$ is the most mass efficient and route $B_{\text{entire one-pot}}$ is the most cost effective synthetic pathway to the bis-THF alcohol. In comparing these two synthetic pathways a 74% decrease in cE-factor and 43% increase in process cost from route $B_{\text{entire one-pot}}$ to route $A_{\text{entire step-by-step}}$ procedure was found. The reduction in cE-factor accompanied by an increase in cost makes route $A_{\text{entire step-by-step}}$ an outlier. This suggests that the synthesis cost predominantly depends on individual material pricing and commercial supplier i.e., if one material is very expensive that alone could make the route more expensive than another route using a larger number of materials. Additionally, both route C procedures showed the highest cost and cE-factor.

Since a goal of green chemistry is to gain financial returns whilst minimizing waste generation, the relationship of cE-Factor versus cost for all synthesis routes was graphically represented in Figure 11.

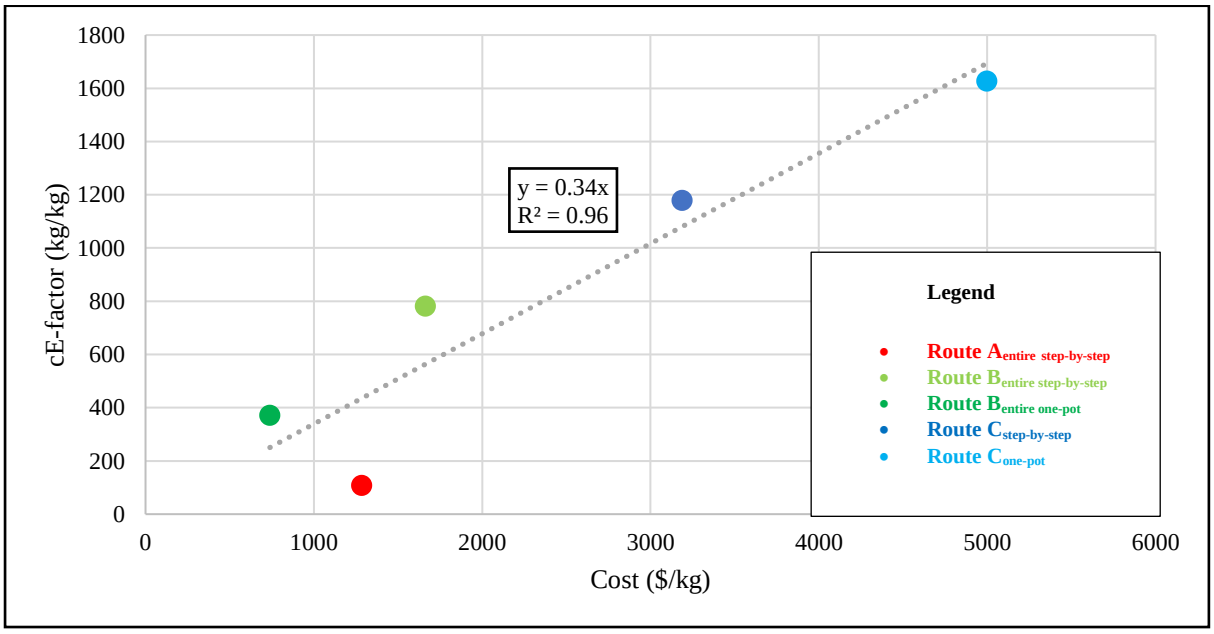


Figure 11: cE-factor vs cost for the synthesis of 1 kg bis-THF alcohol

A strong positive correlation was found between cE-factor and cost with a high regression coefficient (R^2 value) of 0.96. This suggests that reduction in waste generation has a strong economic incentive. A

similar relationship was found between E-Factor (90% solvent recovery) and cost with an R^2 value of 0.95 as well as sE-Factor and cost with an R^2 value of 0.84, the graphical representations of which are illustrated in Appendix F.6.

Further Figure 11 shows that route $B_{\text{entire one-pot}}$ procedure has a 53% cE-factor and 56% cost reduction compared to route $B_{\text{entire step-by-step}}$ procedure, which is in alignment with Hayashi's (2015) statement that these types of procedures are 'pot- economic' with less use of chemical materials and waste generation. The superior mass and economic performance of route $B_{\text{one-pot}}$ procedure was owed to its optimized reaction conditions and reduced material masses, making it more sustainable of the two route B procedures. Even though one-pot operations are highly beneficial, there still remains the challenge of obtaining an enantiomerically pure product.

In contrast, route $C_{\text{step-by-step}}$ showed a 38% cE -factor reduction and 57% cost reduction compared to route $C_{\text{one-pot}}$ procedure suggesting that route $C_{\text{step-by-step}}$ procedure was more eco-efficient than route $C_{\text{one-pot}}$ procedure. Unlike route $B_{\text{entire one-pot}}$ procedure, route $C_{\text{one-pot}}$ procedure did not report any prior selection of optimum reaction conditions and required higher material masses. The one-pot procedure of route C formed part of the step-by-step synthetic pathway to reduce the number of synthesis steps, making the process simpler and more convenient but requiring a larger quantity of chemical materials and hence greater waste generation.

Overall Figure 11 showed that route $B_{\text{entire one-pot}}$ procedure was the most eco-efficient synthetic pathway demonstrating significant cost saving and reduced environmental waste burden compared to the other synthetic pathways.

5.1.11 Summary of experimental procedures

Table 13 below, illustrates a summary of chemical materials, operating temperatures, yields, costs and E-factor values for each synthetic pathway assessed separately.

Table 13: Key aspects in synthetic routes A, B and C to bis-THF alcohol

	Route A		**Route B**			**Route C**	
Synthesis	Subordinate	Step-by-step	Subordinate	Step-by-step	One-pot	Step-by-step	One-pot
Catalyst	Tetramethyl piperidinyloxyl (TEMPO)(1 mol%)	(S)-Diphenylprolinol (D2PM) (3 mol%) Palladium on activated charcoal (Pd/C) (7 mol%)	None	Palladium on activated charcoal (Pd/C) (2 mol%) Porcine Pancreas Lipase (PPL) (5 mol%)	Palladium on activated charcoal (Pd/C) (2 mol%) Porcine Pancreas Lipase (PPL) (8 mol%)	N,N-Dimethylformamide (DMF) (11 mol%)	N,N-Dimethylformamide (DMF) (23 mol%)
Solvent	Diethyl ether (Et_2O) Isopropyl Acetate (iPrOAc)	Toluene (PhMe) Tetrahydrofuran (THF) Methanol (MeOH) Acetic acid 2-Propanol Polyethylene Glycol (PEG_{400})	Ethyl Acetate (EtOAc) Methanol (MeOH)	Methyl tert-butyl ether (MTBE) Cyclohexane (c-hexane) Ethyl Acetate (EtOAc)	Methyl tert-butyl ether (MTBE) Cyclohexane (c-hexane) Ethyl Acetate (EtOAc)	Methyl Tetrahydrofuran Cyclopentyl Methyl Ether (CPME) Dichloromethane (DCM) Toluene (PhMe) Tetrahydrofuran (THF) Ethyl Acetate (EtOAc)	Methyl Tetrahydrofuran Cyclopentyl Methyl Ether (CPME) Dichloromethane (DCM) Toluene (PhMe) Tetrahydrofuran (THF) Ethyl Acetate (EtOAc)
Temperature (℃)	Reaction: 0 – 25 Distillation: 80	Reaction: 0 -35 Distillation: 72 - 197	Reaction: 0 -25 Distillation: 105	Reaction: 25 Chromatography: 22	Reaction: 25 Chromatography: 25	Reaction: -5 – 105 Distillation: 80	Reaction: -8 – 108 Distillation: 106 Distillation: 80
Time (hours)	Reaction: 9.50 Distillation: 1	Reaction: 54.50 Distillation: 4.50	Reaction: 30 Distillation: 1	Reaction: 44 Distillation: 22	Reaction: 72 Distillation: 72	Reaction: 51.20 Distillation: 44	Reaction: 46.05 Distillation: 2 Distillation: 44
No. Chemical materials	12	20	6	9	9	17	21
Overall yield (%)	67	81	67	36	35	50	44
Cost ($)	140	1487	349	1663	739	3308	4673
Traditional E-factor ($kg.kg^{-1}$)	3.10	9.29	15.76	61.41	22.49	124.85	273.37

5.1.12 Comparing average material compositions of bis-THF alcohol to API benchmark

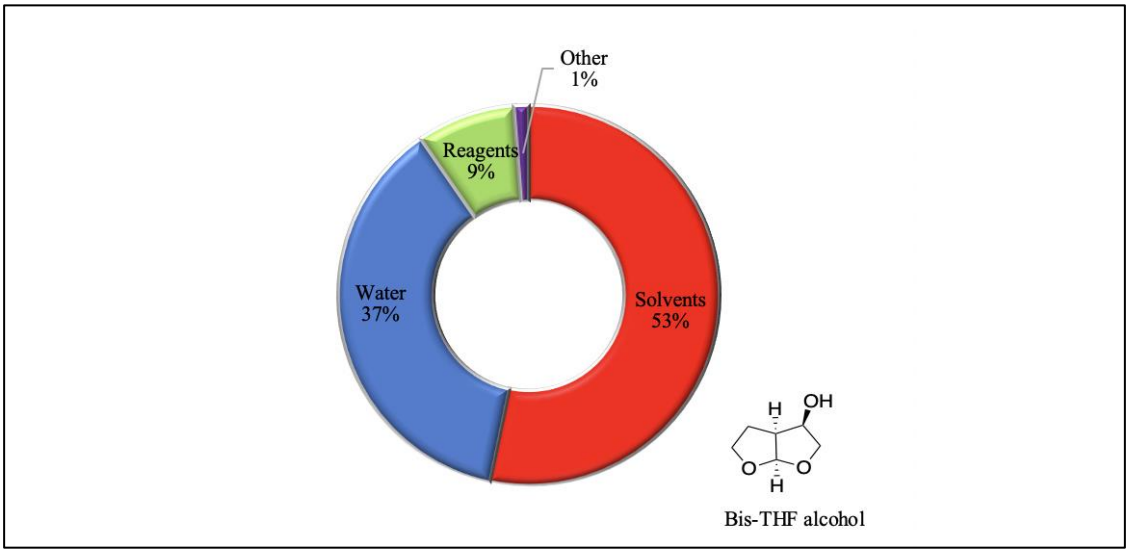


Figure 12: Combined material averages of routes A, B and C to manufacture 1 kg bis-THF alcohol

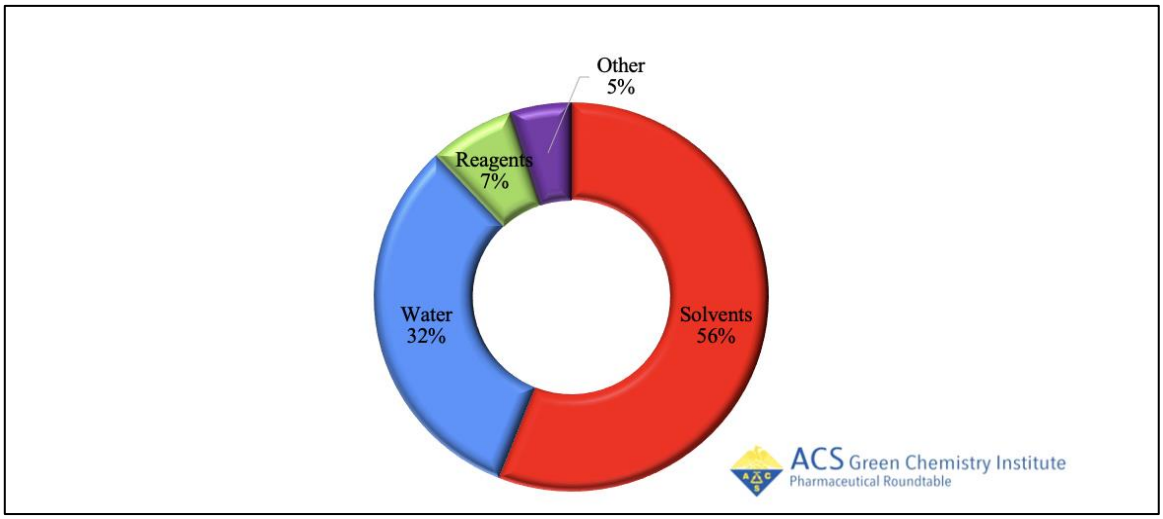


Figure 13: Process mass intensity benchmark 2008 (ACS Green Chemistry Institute, 2014)

Figure 12 illustrates the average materials required per category in synthesizing the bis-THF alcohol via routes A, B and C. The 'other' category represents the average raw materials used, in routes A, B and C. Figure 13 shows the benchmarked material percentages of the ACS GCIPR in manufacturing an API which represents the average across 10 pharmaceutical companies. Comparison of Figure 12 and Figure 13 reveals that current routes to bis-THF alcohol entail 3% less solvents but 5% more water as an environmentally benign solvent alternative. The combination of reactants and other materials used in current bis-THF alcohol routes only differed by 2% compared to ACS benchmark values.

5.2 Green Scorecards - iGAL™

The iGAL™ methodology is based on a data set of 64 synthetic API pathways comprising 703 synthesis steps at different development stages from 12 pharmaceutical companies (Roschangar et al., 2018). Table 14 represents the mean findings obtained from the data set along with the average bis-THF alcohol synthetic route findings at an early development stage. The average cE-factor of the bis-THF alcohol was used to find the closest correlation to the average iGAL™ cE-factors and thus the development stage for the bis-THF alcohol to be assessed.

Table 14: Mean findings for 64 API molecules at various drug manufacturing stages as reported by Roschangar et al., (2018) and findings for bis-THF alcohol averages of routes A,B and C.

	Development stage	**FMW (g/mol)**	**cE-factor (kg/kg)**	**iGAL™**
64 API's	Early	451	709	155
	Late	464	352	160
	Commercial	449	155	155
Bis-THF alcohol	Early	130	810	145

Using the data from Table 14, the Green Scorecard for API synthesis at an early development stage was developed with a mean FMW of 451g/mol and mean cE-factor of 709. The bis-THF alcohol however forms part of a complete API structure (i.e., an API sub-unit) with an FMW of 130.14g/mol and an average cE-factor of 810, both of which are far from the average early development score card values used. Owing to the small FMW and large cE-factors of the bis-THF alcohol not returning readings on the Green Scorecards, the traditional E-factors were used (which assumes 90% solvent recovery and does not consider process water) in place of the cE-Factor values.

From the commercial data in Table 14 the iGAL™ mathematical Equation E7 was created:

$$\text{iGAL} = 0.344 \times \text{FMW}\left[\frac{\text{kg waste}}{\text{kg API}}\right] \qquad (E7)$$

The constant 0.344 was derived from the data set of the 64 drug molecules where the mean for commercial manufacturing stage cE-factor (155) was divided by the mean FMW (449) and multiplied by 1000. Using iGAL™ the greenness of the process relative to industrial averages (RPG) across development phases (early, late and commercial) could be then be determined using Equation E8:

$$\text{RPG} = \frac{\text{iGAL}}{\text{cE factor}} \times 100\% \qquad (E8)$$

After the RPG score is obtained, the RPG matrix (Table 14) is used to rate the synthetic pathway at the respective development stage. Finally, the innovation impact (process improvements) may then be determined.

Table 15: iGAL™ RPG matrix for green API manufacturing at various development stages (Roschangar et al., 2018)

Percentile	**Code**	**Rating**	**Minimum relative process greenness (RPG) rating**		
			Early development	**Late development**	**Commercial**
90%		Excellent	66%	146%	222%
70%		Good	48%	103%	168%
40%		Average	29%	59%	113%
		Below Average			
RPG		Industrial Average	35%	73%	131%

Bis-THF alcohol iGAL™ and Green Scorecards

The Green Scorecards for all possible route A, B and C combinations and comparisons are illustrated in Appendix H.2, with a summary of the scorecard results represented in Table 16 and Table 17. The Green Scorecard requires input metrics FMW, cE-Factor, CP, number of synthesis steps and outputs process ideality (I), RPG, process improvements and percentage waste reduction thereby reflecting the mass-efficiency of the synthesis. Since all the synthetic routes lead to the same target molecule (bis-THF alcohol), the FMW remained constant at 130.14g/mol and the early development waste target (iGAL™) remained constant at 45 (i.e., 0.344 × 130.14g/mol = 45) for all routes. This compares well with the average early stage industrial drug manufacturing benchmark.

Table 16: Green Scorecard input and output data per synthesis route to bis-THF alcohol

		Routes				
		$A_{entire\ step\text{-}by\text{-}step}$	**$B_{entire\ one\text{-}pot}$**	**$B_{entire\ step\text{-}by\text{-}step}$**	**$C_{one\text{-}pot}$**	**$C_{step\text{-}by\text{-}step}$**
Input data	E-factor	14.54	89.69	126.77	273.37	124.84
	Complexity (i.e.,Σ construction steps)	4	4	4	2	2
	No. of synthesis steps	5	2	5	3	5
Output data	RPG	308%	50%	35%	16%	36%
	Performance versus industrial early development benchmark (RPG =35%)	9.09 times less wasteful	1.43 times less wasteful	1.01 times less wasteful	2.14 times more wasteful	1.02 times less wasteful
	Green industrial performance percentile	Falls within the top 10%	Falls within the top 30%	Falls within the top 60%	Falls within the bottom 40%	Falls within the top 60%
	Project status and colour code	Excellent	Good	Average	Below average	Average

The 35% RPG benchmark for 'early development' API synthesis obtained from the RPG matrix grid (Table 15) was required in determining the results represented in Table 16. Additionally, Equation E8, was used in determining the RPG for each route, for example route $A_{step\text{-}by\text{-}step}$ RPG is 308% (i.e., $\frac{45}{14.54} \times 100\% = 308\%$), after which its wastefulness versus industrial benchmark was determined (i.e., 308%/ 35% = 9.09 times less wasteful). The RPG matrix grid was then used to determine in which percentile of greenness performance the synthetic pathway falls into. For instance, route $A_{step\text{-}by\text{-}step}$ has an RPG of 308% (>> 66%) and thus falls within the top 10% of green industrial performance and rated 'excellent' in mass- efficiency performance. These assessment steps were conducted for all entire synthetic routes A, B and C the results of which are illustrated in Table 16.

Moreover, Table 16 showed that route $A_{step\text{-}by\text{-}step}$ was the least waste generating synthesis with the lowest E-factor of 14.54 and an 'excellent' synthesis status being 9 times less wasteful than early stage API synthesis benchmark. Conversely, route $C_{one\text{-}pot}$ was found to be the most waste generating synthesis with the highest E-factor of 273.37 and a 'below average' synthesis status being 2.14 times more wasteful than early stage API synthesis benchmark. In between these two E-factor lies route $B_{entire\ one\text{-}pot}$, $B_{entire\ step\text{-}by\text{-}step}$ and $C_{step\text{-}by\text{-}step}$ with E-factor values of 89.69, 126.77 and 124.84 with synthesis status of 'good', 'average' and 'average' respectively. Thus, from the two variations of route B and C procedure, route $B_{entire\ one\text{-}pot}$ and $C_{step\text{-}by\text{-}step}$ procedure had preferred mass-efficiency performance. Further, route $A_{step\text{-}by\text{-}step}$ was the most mass- efficient synthetic pathway to the bis-THF alcohol, with the best performance in process greenness relative to 'early development' industrial averages.

It is important to note that the iGAL^{TM} methodology and Green Scorecard did not determine which material category had the greatest mass contribution to waste generated as well as synthesis cost both of which are required in determining and comparing green and sustainable drug manufacturing. Further, the iGAL^{TM} metric and Green Score card were designed to assess complete API structures and not API sub-units such as the bis-THF alcohol. Despite this, the bis-THF alcohol has been reported to be the most complex and costly moiety in synthesizing complete API ARV structures such as DRV (Moore, Stringham, Teager and Yue, 2017), with numerous synthetic pathways reported in its history. Further, from Table 16 it is evident that synthetic pathways to this sub-unit perform equally as badly as a synthetic pathway to a complete API structure, when considering mass-efficiency, ranking 'average ' and 'below average' in routes $B_{entire\ step\text{-}by\text{-}step}$ and $C_{one\text{-}pot}$ respectively.

5.2.1 Comparison between assessed bis-THF alcohol routes.

Table 17 compares the difference in waste amounts (reflected by E-factor or percentage waste reduction) and overall process improvements between all possible synthetic route combinations to bis-THF alcohol assessed in ascending order of waste reduction.

Table 17: Comparing reduction in waste generation and overall process improvements between bis-THF alcohol synthetic routes.

Routes compared	E-factor reduction (kg/kg)	Waste reduction (%)	Overall process improvements
From $B_{\text{entire step-by-step}}$ to $C_{\text{step-by-step}}$	1.91	2	1%
***From $C_{\text{step-by-step}}$ to $B_{\text{entire one-pot}}$**	**35.16**	**28**	**14%**
From $B_{\text{entire step-by-step}}$ to $B_{\text{entire one-pot}}$	37.08	29	15%
From $C_{\text{one-pot}}$ to $B_{\text{entire step-by-step}}$	146.61	54	19%
From $C_{\text{one-pot}}$ to $C_{\text{step-by-step}}$	148.53	54	19%
From $C_{\text{one-pot}}$ to $B_{\text{entire one-pot}}$	183.68	67	34%
***From $B_{\text{entire one-pot}}$ to $A_{\text{entire step-by-step}}$**	**75.50**	**84**	**259%**
***From $C_{\text{step-by-step}}$ to $A_{\text{entire step-by-step}}$**	**110.50**	**88**	**273%**
From $A_{\text{entire step-by-step}}$ to $B_{\text{entire step-by-step}}$	112.50	89	273%
From $C_{\text{one-pot}}$ to $A_{\text{entire step-by-step}}$	258.50	95	292%

* **bold text comparing most mass efficient route A, B and C**

From all the possible route combinations, Table 17 shows that the greatest amount of waste reduction was found between route $C_{\text{one-pot}}$ and $A_{\text{entire step-by-step}}$ with an E-factor reduction of 258.50 kg. Oppositely, between route $C_{\text{step-by-step}}$ and $B_{\text{entire step-by-step}}$ represented the smallest variation in waste generation with a 2% waste reduction.

In comparing the most mass-efficient procedures of each synthesis route (i.e., $A_{\text{entire step-by-step}}$, $B_{\text{entire one-pot}}$ and $C_{\text{step-by-step}}$), the largest waste reduction was found from route $C_{\text{step-by-step}}$ to route $A_{\text{step-by-step}}$ with a 110.50 E-factor reduction and 273% process improvement. The second greatest waste reduction was from route $B_{\text{entire one-pot}}$ to route $A_{\text{entire step-by-step}}$ with a 75.50 E- factor reduction and a 259% process improvement. The smallest waste reduction was found from route $C_{\text{step-by-step}}$ to route $B_{\text{entire one-pot}}$ with a 35.16 E-factor reduction with a 14% process improvement. The E-factor reductions between all routes are mainly attributed to mass of solvent and water used.

5.3 Solvents

From, Figure 14 it is evident that solvents are the primary waste generators in both route B and route A procedures, contributing 45%, 84% and 96% of the total mass in route $A_{step\text{-}by\text{-}step}$, $B_{one\text{-}pot}$ and $B_{step\text{-}by\text{-}step}$ procedure respectively. Even though water is the greatest waste contributor in route C procedures, solvents still have a significant mass contribution of 41% and 46% in route $C_{one\text{-}pot}$ and $C_{step\text{-}by\text{-}step}$ respectively. The quantity of solvents and water used were approximately in alignment with ACS pharmaceutical benchmarked values (Figure 14) except for route B that did not use water as a material component.

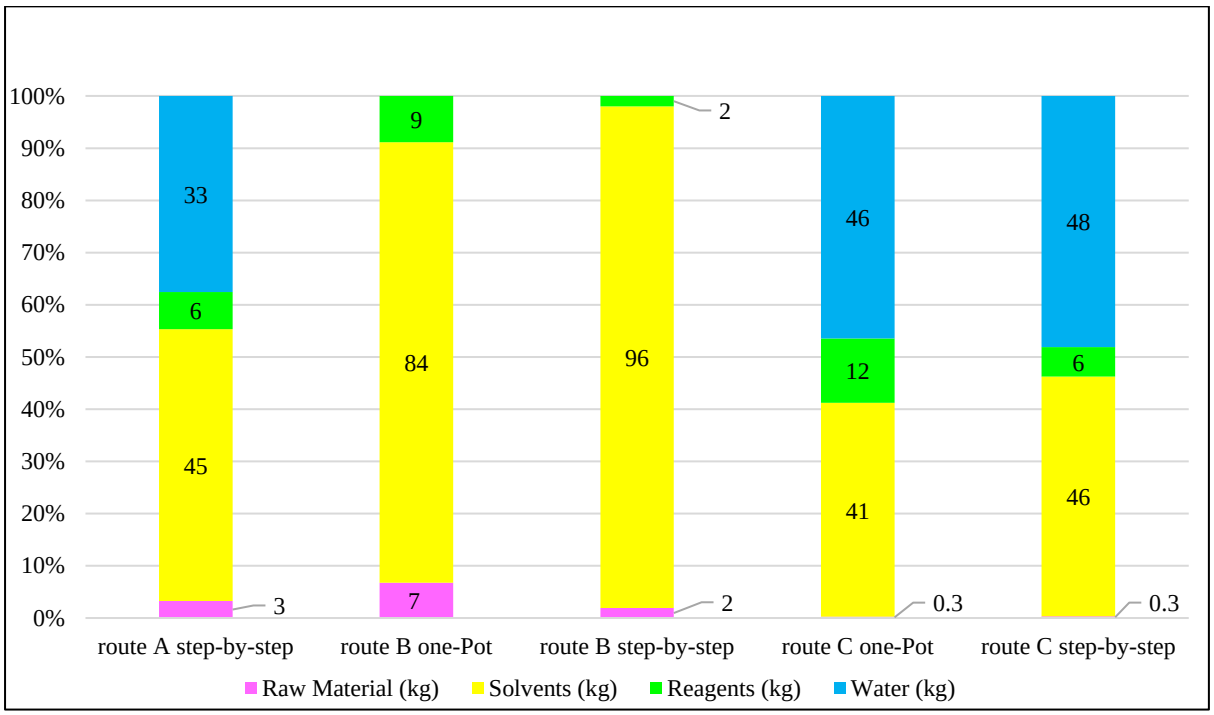


Figure 14: Chemical material composition for entire synthetic pathways assessed.

Organic solvents represent the greatest environmental concern and thus hold the greatest potential in reducing waste for all synthetic pathways. Table 18, illustrates the solvent intensities (SI's) for each entire synthetic pathway where the mass of product is 1 kg bis-THF alcohol. The SI's demonstrate the mass quantity of the solvents and not the nature of the solvents used. The most effective way to minimize their environmental impact is to reduce the quantity and increase the quality used. To clarify, the preferred solution for hazardous and toxic solvents is to replace them with greener alternatives and not to reduce the amounts used.

Table 18: Solvent intensity for entire synthetic pathways A, B and C to 1 kg bis-THF alcohol

Synthesis route	**SI** $\left(\frac{kg\ solvent}{kg\ product}\right)$
$A_{\text{entire step-by-step}}$	48
$B_{\text{entire step-by-step}}$	509
$B_{\text{entire one-pot}}$	89
$C_{\text{step-by-step}}$	539
$C_{\text{one-pot}}$	662

From Table 18, it is apparent that route $C_{\text{one-pot}}$ had the greatest solvent intensity at 662 and route $A_{\text{entire step-by-step}}$ had the lowest at 48 in producing 1 kg bis-THF alcohol, that is a large solvent mass difference of 614 in manufacturing the same amount of the desired product. In comparing the two lowest SI values, route $A_{\text{step-by-step}}$ demonstrated 46% lower solvent mass than route $B_{\text{one-pot}}$, making route $A_{\text{step-by-step}}$ procedure the best preforming route from a solvent mass perspective. Further in comparing the different processes within the same routes, an 83% SI reduction was found from route $B_{\text{entire step-by-step}}$ to route $B_{\text{entire one-pot}}$ procedure. The large solvent reduction was attributed to the step-by-step procedure performing purification of intermediates between each synthesis step and the one-pot procedure under optimized conditions (as previously discussed in Scheme 4 and Scheme 5). This runs in parallel with one-pot procedures being less mass and energy intensive, further indicating the importance of process optimization. The opposite was found between the route C procedures with route $C_{\text{step-by-step}}$ having an 18.5% lower SI compared to $C_{\text{one-pot}}$ procedure. This was attributed to the reaction conditions not being optimized, higher solvent quantities required and the one-pot procedure only forming part of the synthesis and not the complete synthetic pathway as found in route $B_{\text{one-pot}}$ procedure (as previously discussed in Scheme 7).

Despite route $A_{\text{entire step-by-step}}$ having the lowest SI, there were a larger variety of solvents used which were more hazardous, with unfavourable colour and GHS ratings as compared to route B and C procedures (Table 19). The SI metric does not account for the nature of the solvent materials and similarly the cE-Factor metric assigns all waste types, including solvent waste, the same impact weighting.

Assessing the nature of the solvent is more complex and highly dependent on the evaluation system selected. As previously described the GSK solvent guide employs a traffic light inspired colour coding, based on multiple sustainability factors, to determine solvent status (Table 5). Conversely, the Green Motion™ tool utilizes a pictogram system and categorizes solvents into various classes (supercritical, petrochemical, renewable and carcinogenic, mutagenic and reprotoxic (CMR)) where higher penalty points are assigned to more hazardous solvents as illustrated in Table 6 (concept 2 and 3). For example, the GSK solvent guide (Section 3.3) assigns MeOH a light green colour code suggesting it as a recommended or reasonable solvent and assigns DMF a red colour code suggesting a highly hazardous solvent with health and safety concerns that mean it should be banned. In contrast the Green Motion™ solvent assessment assigns solvent MeOH the same pictograms and CMR category as DMF, receiving the same penalty points and undesired solvent rating. Further, many of the solvents used in routes A, B and C were not available in the Green Motion™ tool with alternative greener solvent options not recommended. Since different evaluation systems lead to inconsistencies in interpretation and the bis-THF alcohol predominantly found in the pharmaceutical industry, the GSK solvent guide was selected in qualitatively assessing the relative greenness of solvents used in each synthetic pathway.

It is important to note that the GSK solvent guide only considers individual solvents. Alder *et al.*, (2016), in contrast, state that complex solvent mixtures and their recovery should be dealt with on a case-by-case basis. Thus, solvents assessed in each of the bis-THF alcohol synthetic pathways were considered as single entities. Assessing the nature of the solvents, their cost implications and finding greener alternatives aids in determining the most sustainable synthetic pathway to the bis-THF alcohol.

Table 19 illustrates the solvent masses and respective solvent profiles per synthetic pathway, as obtained from the Excel Spreadsheets and classified by GSK solvent guides. The boiling or melting point for each solvent is shown owing to their significant influence in determining the solvent's colour profile. The quantity of each solvent is illustrated underneath the boiling or melting point and demonstrated for both the step-by-step and one-pot procedures in routes B and C. The total number of solvents used for the entire synthesis route (subordinate and main) are provided in the last row of the table. Since water was assigned its own material category (in calculating E-factors) and has negligible environmental impact, it was eliminated from Table 19 and the solvent analysis.

Table 19: Assigned solvent colour profiles, melting or boiling points as categorized by GSK solvent guide and solvent masses per synthesis route (Alder et al., 2016).

Entire Route A		**Entire Route B**		**Entire Route C**
Subordinate	**Step-by-step**	**Subordinate**	**Step-by-step / One- pot**	**Step-by-step / One- pot**
Isopropyl acetate (iPrOAc) (89°C) 24 kg	2-Propanol (82°C) 5 kg	Ethyl acetate (EtOAc) (77°C) 115 kg	Ethyl acetate (EtOAc) (77°C) 61 kg / 23 kg	Ethyl acetate (EtOAc) (77°C) 24 kg / 24 kg
Di-ethyl ether (Et_2O) (35°C) 3 kg	Polyethylene glycol (PEG_{400}) (mp 8°C) 1 kg	Methanol (MeOH) (65°C) 103 kg	Methyl tert-butyl ether (MTBE) (55°C) 359 kg / 33 kg	Toluene (PhMe) (111°C) 250 kg / 230 kg
	Methanol (MeOH) (65°C) 2 kg		Cyclohexane (c-hexane) (81°C) 89 kg / 33 kg	Cyclopentyl methyl ether (CPME) (106°C) 36 kg / 66 kg
	Acetic acid (118°C) 0.7 kg			Methyl tetrahydrofuran (Me-THF) (78°C) 34 kg / 39 kg
	Toluene (PhMe) (111°C) 9 kg			Tetrahydrofuran (THF) (65°C) 27 kg / 27 kg
	Tetrahydrofuran (THF) (65°C) 2 kg			Dichloromethane (DCM) (40°C) 168 kg / 276 kg
8		**4**		**6**

Two important factors that play a role in assessing how good or bad a solvent is, is the flammability and the CMR status of the solvent. Flammability is related to the boiling point of the solvent where orange to red colour rankings and high penalty points are assigned to solvents with boiling points lower than 80°C and higher than 120°C. This means that, for example, solvent EtOAc (b.p. 77°C) scores lower than iPrOAc (b.p. 89°C) although this difference is minimal in practice it is incomparable with the

enormous problems associated with Et_2O (b.p. 35℃) which is prohibited on industrial scale. CMR status is related to each solvent's health statement and code (obtained from PubChem, (2019)) as discussed in the paragraphs to follow.

Route $A_{step\text{-}by\text{-}step}$ has a total solvent mass of 20 kg with toluene (PhMe) having the greatest contribution at 45% (9 kg) followed by 2-propanol at 25% (5 kg). Potential greener replacements for PhMe, as per GSK solvent guide, were anisole and *p*-xylene. The health statements associated with PhMe are H225 for high flammability and H304 for fatality when swallowed. Even though 2-propanol is a green solvent, greener alternatives including 1-propanol, heptanol or ethylene glycol may still be considered. The health statement associated with 2-propanol was H225 for high flammability, H319 for eye irritation and H336 for drowsiness and dizziness making it more appropriate to consider applicable greener alternatives. Even though PEG_{400} solvent contributed a relatively small quantity of 5%, it was significantly less hazardous relative to the other route A solvents due to its negligible vapour pressure and VOC emissions, allowing it to remain stable under ambient operating conditions (Vafaeezadeh and Hashemi, 2015). Additionally, PEG_{400} has a low toxicity and is considered biologically inert and safe (Sheftel, 2000), further complying with the 5^{th} and 12^{th} principle of green chemistry. Furthermore, PEG_{400} shows good performance as a low molecular weight solvent with recyclable reaction medium in catalytic and enzymatic reactions (Chen, Spear, Huddleston and Rogers, 2005; Zhong, Dou and Wang, 2013).

In route $A_{subordinate}$ the total solvent mass was 27 kg from which 89% was attributed to the iPrOAc solvent (24 kg). The iPrOAc solvent was used in large mass excess compared to the highly hazardous Et_2O (mass ratio of Et_2O : iPrOAc as 1 : 8). Thus, iPrOAc was assigned a green colour ranking for its low toxicity and easy biodegradability (Alder, Hayler, Henderson, Redman, Shukla, Shuster and Sneddon, 2016) and was further considered a green solvent, with no need for solvent replacement. The solvent complies with the 4^{th}, 5^{th} and 10^{th} principle of green chemistry which are in alignment with increased mass and energy efficiency. Further, from an industrial perspective, iPrOAc has a low water solubility (2.90g per 100g water at room temperature (McConville, 2003)) which offers an advantage when considering downstream process operations. Additionally, it complies with the 3^{rd} and 6^{th} principle of green engineering in accordance with health and safety operations. Further, Et_2O was assigned a dark red 'highly hazardous' solvent ranking which will not be scaled up to industrial scale. Thus, according to GSK's solvent sustainability guide Et_2O has major environmental, health and safety issues and should be replaced by greener alternatives. Potential greener ether solvents to Et_2O all of which comprise fewer environmental and health hazards include methyl-THF, ethyl *tert*-butyl ether (ETBE), dimethyl isosorbide, cyclopentyl methyl ether (CPME) or may be replaced by iPrOAc as an extraction solvent as previously discussed (Scheme 1).

In considering route B, the total solvent mass used in route $B_{subordinate}$ was 218 kg where each of the solvents were used in relatively equal amounts (mass ratios EtOAc : MeOH at 1.12 : 1). The total solvents used in route $B_{step\text{-}by\text{-}step}$ procedure was 509 kg where MTBE contributed the largest amount (358 kg) followed by c-hexane (89 kg) and the remainder being EtOAc (61 kg). The top three greener solvent replacements for MeOH are heptanol, ethylene glycol or octanol. The total solvent mass used in route $B_{one\text{-}pot}$ procedure was 89 kg where the solvent types were used in fairly equal amounts (mass ratio MTBE : c-hexane : EtOAc was 1.43 : 1.46 : 1). An advantage of route B was the consistent use of the three solvents mentioned throughout the synthetic procedure allowing for ease of solvent recycling. A potential greener solvent for EtOAc was glycerol diacetate or isobutyl acetate. Further, a greener solvent alternative for c-hexane was iso-octane. The health statements associated with c-hexane are H304 and H410 which are associated with being fatal if swallowed and further toxicity to aquatic life. Further, as illustrated in Table 19, the entire route B procedure was found to be most desirable using minimum number of four solvents, with favorable EHS characteristics and solvent colour profiles.

In route $C_{step\text{-}by\text{-}step}$ the total solvent mass was 539 kg from which PhMe (250 kg) and DCM (168 kg) collectively contributed more than three-quarters of the solvent mass. DCM contributes 31% of the total solvent use with major issues from a waste, environment, health and safety perspective and was thus assigned a red colour ranking. Since it was ranked as a hazardous solvent it puts route $C_{step\text{-}by\text{-}step}$ at a high disadvantage as a green method to produce bis-THF alcohol. DCM has a high volatility (high VOC emissions with P_{vap} = 57.30kPa at 25℃ (National Institute of Standards and Technology, 2019)) making it an acute inhalation and skin absorption hazard. Additionally, it has great difficulty in being incinerated as a waste product (Alder, Hayler, Henderson, Redman, Shukla, Shuster and Sneddon, 2016). Similarly, in route $C_{one\text{-}pot}$ procedure the total solvent mass was 662 kg from which PhMe (230 kg) and DCM (276 kg) contribute three quarters to the total solvent mass. Both these solvents hold the same concerns and demerits as the route $C_{step\text{-}by\text{-}step}$ procedure. It is important to note that the DCM solvent is associated with health statement H351that poses carcinogenic concerns as well as a low boiling point of 40℃ that may lead to handling difficulties at a commercial stage. Further, DMF was used in catalytic quantities (2 drops) and is thus not categorized as a solvent however the use of DMF entails the health phrase H360 thus having dangers regarding reproductive toxicity. A merit of the both route C procedures lies in the use of less common, bio-based ethers CPME and Me-THF that have lower water solubility and less hazardous properties to classical ether solvents (Sheldon, 2019), even if in smaller amounts.

Over and above solvents being the greatest health hazards and mass contributors in organic synthesis; solvent costs significantly impact the sustainability and the selection of the pathway. Thus, the cost associated with total solvent use for each of the synthetic pathways demonstrated that:

- In route A, 39% of the total synthesis cost was attributed to solvents,

- In route $B_{step\text{-}by\text{-}step}$ and route $B_{one\text{-}pot}$ procedures, 81% and 30% of the total synthesis costs are attributed to solvents respectively and
- In route $C_{step\text{-}by\text{-}step}$ and route $C_{one\text{-}pot}$ procedures, 41% and 39% of the total synthesis cost are attributed to solvents respectively.

Thus, route $B_{one\text{-}pot}$ procedure had the cheapest solvent cost contribution in manufacturing of 1 kg bis-THF alcohol. Further, route $B_{one\text{-}pot}$ cost associated with solvent re-cycling and disposal was expected to be minimal owing to the minimum number of green solvents used with few associated issues.

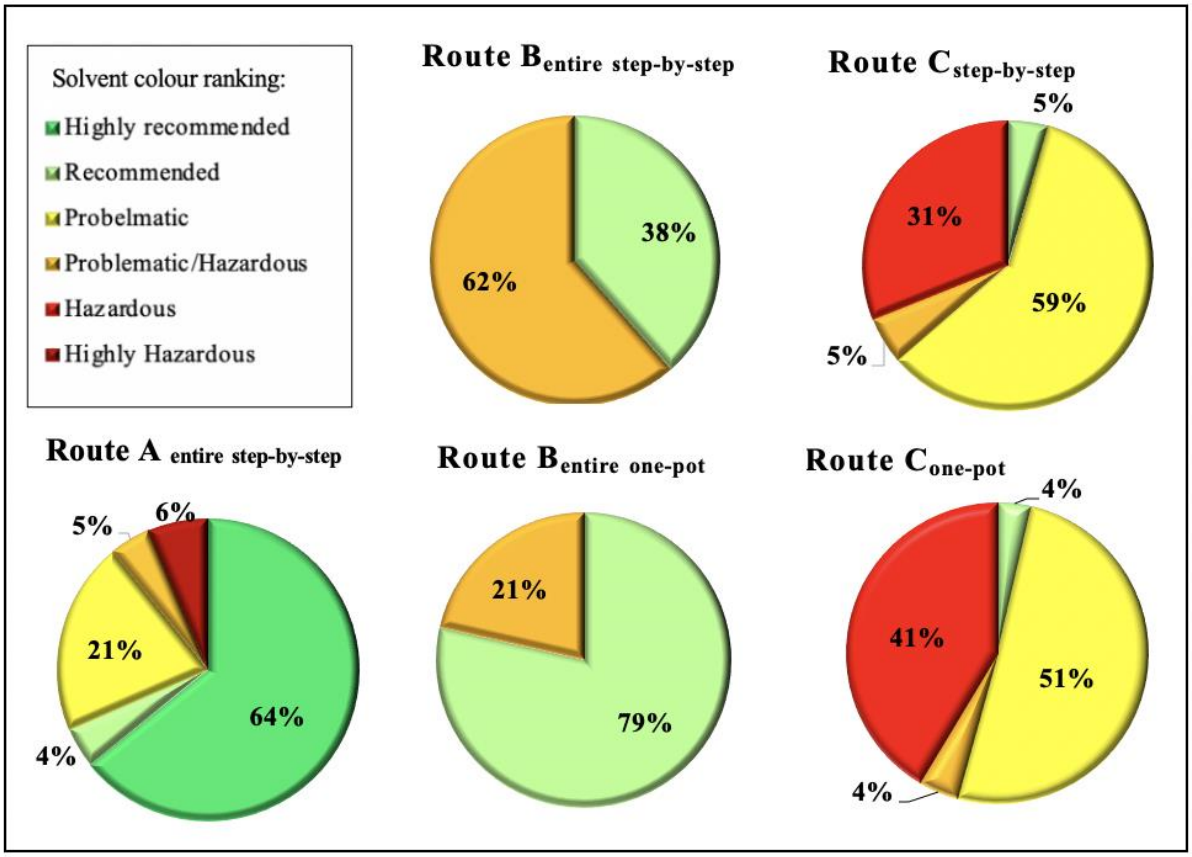


Figure 15: Colour ranking and mass percentage contribution of solvents used per entire synthetic procedure to 1 kg bis-THF alcohol.

Figure 15 above compactly illustrates the mass percentage of solvents used, with respect to the GSK solvent profiles, for each synthetic pathway assessed.

In route $A_{entire\ step\text{-}by\text{-}step}$ procedure 6% of solvents used were highly undesired, 30% were acceptable and 64% were preferred. The highly undesired solvents are assigned a dark red colour implying that they are associated with severe human and environmental health issues. The demerit of route $A_{entire\ step\text{-}by\text{-}step}$ was demonstrated in the array of solvents used from all solvent ranking categories. This may cause issues at later life-cycle stages as solvent mixtures require more expensive recycling, waste treatment

or incineration disposal. This makes route A an unfavourable synthetic pathway comparative to route B.

At a glance it was evident that route $B_{\text{entire one-pot}}$ procedure made the greatest effort in using green solvent alternatives with close to 80% of the total solvents used having a recommended solvent colour ranking. Further, route $B_{\text{entire one-pot}}$ procedure depicts a 41% reduction in hazardous solvent use when compared to route $B_{\text{entire step-by-step}}$ procedure. The large reduction in hazardous solvent use was primarily attributed to the elimination of chromatographic purification (c-hexane/EtOAc) of intermediates at each synthesis step. This in turn led to a 51% decrease in solvent cost which was in alignment with green chemistry being both cost effective and environmentally friendly.

Both route $C_{\text{step-by-step}}$ and route $C_{\text{one-pot}}$ procedure consist of ≥95% hazardous and problematic solvents with similar cost contributions (~40% of total synthesis cost). However, in comparing the two route C procedures, route $C_{\text{step-by-step}}$ showed a 10% reduction in hazardous solvent use and 2% increase in greener solvent alternatives compared to the one-pot procedure. Further, it was expected for both route C procedures to possess greater waste, cost, environmental, health and safety issues compared to route A and B, making it the most undesired bis-THF alcohol pathway owing to its large excess of solvent quantities used (> 500 kg solvent per 1 kg target compound).

Solvents assigned dark and light green colour ranking are commonly associated with few issues in terms of environmental impact, waste disposal, human health and safety. Thus, in comparing the best performing routes in A, B and C it was found that route $B_{\text{entire one-pot}}$ uses the largest quantity of green solvents at 79% of total solvent mass, followed by route A at 66% and lastly by route $C_{\text{step-by-step}}$ at a low 5%. This suggests that route $B_{\text{entire one-pot}}$ procedure has the smallest environmental footprint.

5.4 Green Motion™

5.4.1 Adjustments for Green Motion™ tool

Green Motion™ was designed as an in-house formulation tool for the flavour and fragrance industry from which certain assessment criteria may need to be adapted in considering API compounds (Phan, Gallardo and Mane, 2015). For example, using raw materials from natural origins is rewarded because it is important in the flavour and fragrance industry. In contrast, pharmaceutical companies are more concerned with the cost of the raw material than its natural or synthetic status. Nevertheless, most environmentally friendly criteria considered in Green Motion™ that are of equal importance in the pharmaceutical industry include:

- Number of solvents used,
- Extraction methods using solvents that provide the best reaction yields,
- Reduced waste production,
- Minimized energy consumption,
- Mild operating conditions (low temperatures and atmospheric pressure) and
- Easily recycled solvents.

These criteria fall within Green Motion concepts 3, 4, 5, 6 and 7 namely the 'reaction', 'process', 'hazard and toxicity' and 'waste' categories.

In making the Green Motion™ tool more relevant for assessing the bis-THF alcohol synthetic methods, adjustments to the original Green Motion™ requirements were made. Such adjustments include eliminating the 'raw material' concept owing to its minimal importance in pharmaceuticals, and the removal of the 'solvent' concept to avoid conflicting interpretations with the GSK solvent guide (Section 3.3). An additional adjustment is the use of the sE – factor values in place of the standard E-factor or cE-Factor values. The use of cE-Factor values returned a Green Motion™ 'waste' category score of zero for all synthetic pathways. Since 60 - 90% of the cE-Factor values are attributed to solvents and process water, the sE-Factor was selected as the alternative waste measure as it excludes solvents and process water by definition (Roschangar *et al.*, 2018). The use of the sE-factor values returned a numerical score other than zero for the Green Motion™ 'waste' category for all synthetic pathways. This indicates that the sE-Factor values fall within the Green Motion™ penalty score range.

Furthermore, the Green Motion™ tool focuses on processes at pilot scale rather than bench scale. Many of the laboratory techniques reported in the bis-THF alcohol experimental procedures were not presented as Green Motion™ selection options. The laboratory scale operations were then assumed at a pilot scale where an appropriate option presented in the 'process' concept was selected. Table 20 shows the experimental technique with its Green Motion™ counterpart.

Table 20: Laboratory techniques and corresponding Green Motion™ counterpart

Experimental laboratory technique	**Green Motion™ equivalent**
Distillation under nitrogen atmosphere	Atmospheric distillation
Reduced pressure and concentration filtered in vacuo	Reactions not under pressure
Reaction mixtures heated in a water bath to temperatures ≤ 100 ℃	Steam at 1 bar
Reaction mixtures heated in an oil bath to temperatures > 100 ℃	Oil
Reaction mixtures separated into organic and aqueous layers	Liquid-liquid extraction
Chromatographic techniques, rotary evaporation and continuous extraction	Other - energy intensive method

Adopting the Green Motion™ tool at an early assessment stage is fundamental in acknowledging the environmental, safety and health impact values of chemical reactions, process operations and waste generation of each innovative bis-THF alcohol synthetic pathway assessed. In comparing the synthetic pathways, the tool communicates the efforts made to minimize environmental impacts via fundamental concepts. Unlike the iGAL™ and Green Scorecard assessment that requires a cost defined starting point, the Green Motion™ tool focuses predominantly on the origin of the raw materials. Since the ASM's of the synthetic pathways were found using the < 100\$/mol definition by Roschangar *et al.*, (2018), the Green Motion™ scores to the ASM's were included to keep the assessments consistent. This was additionally done to ensure that the ASM's environmental impacts were not neglected and for a consistent starting point to exist between the various assessments.

5.4.2 Green Motion™ scores for synthetic pathways assessed

Figure 16 represents the comparison of route A, B and C per Green Motion™ concept and total score. The original output graphs from the Green Motion™ tool are illustrated in Appendix I.1. As previously stated, the concepts assessed were 'reaction', 'process', 'hazard and toxicity' and 'waste'. The 'hazards and toxicity' category was not illustrated in Figure 16 as it returned a score of zero for all synthetic pathways. This was attributed to all the pathways being multi-step reactions, requiring a large variety of chemical materials each with their own respective GHS pictograms, accumulating the maximum number of penalty points returning a score of zero. The closer the overall Green Motion™ score is to 100, the greener and more sustainable the synthetic pathway.

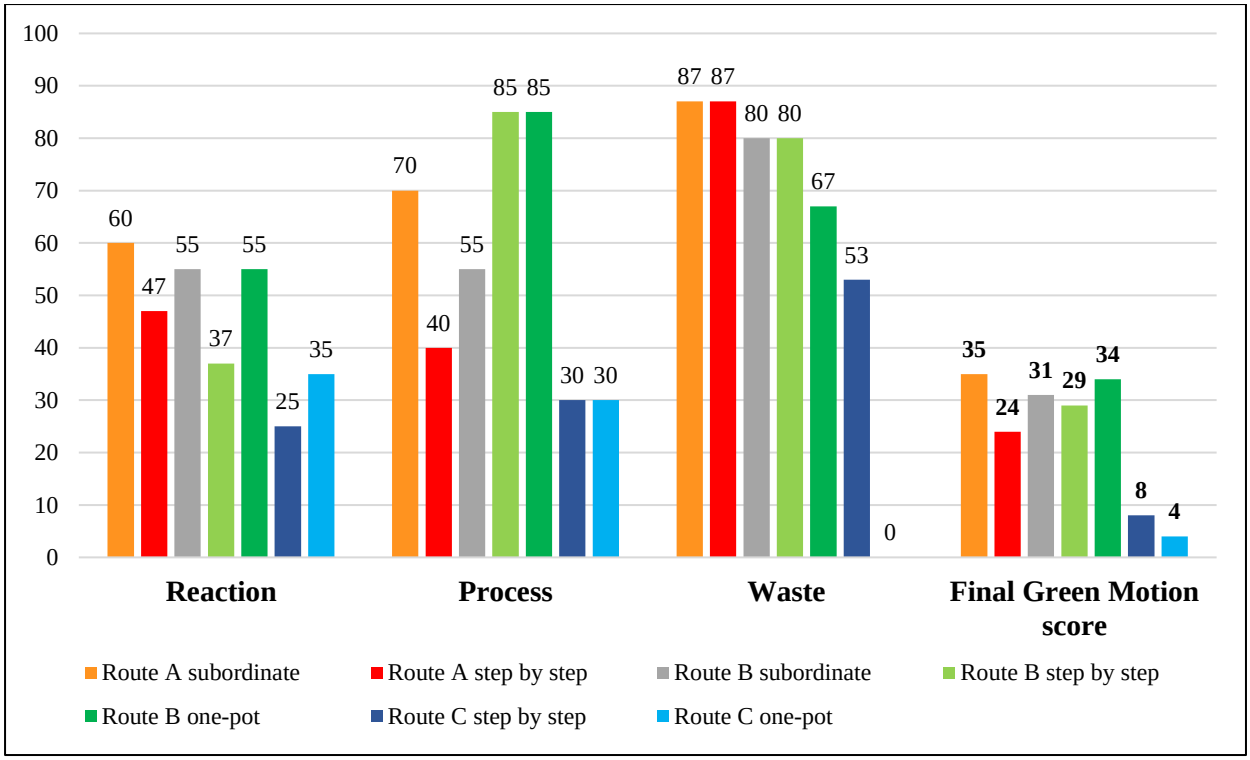


Figure 16: Green Motion™ concept ratings and overall score per synthesis route assessed

Reactions category:

Table 21 illustrates the reaction time and yield, two parameters which were required in assessing the Green Motion™ 'reaction' category. Higher reaction yields and shorter reaction times received fewer penalty points and thus higher Green Motion™ 'reaction' scores.

Table 21: Overall reaction yield and reaction time per assessed synthetic pathway

Synthesis route	Overall yield %	Reaction time (hours)
Route $A_{subordinate}$	67	9.50
Route $A_{step\text{-}by\text{-}step}$	81	54.50
Route $B_{subordinate,}$	67	30
Route $B_{step\text{-}by\text{-}step}$	35	44
Route $B_{one\text{-}pot}$	36	72
Route $C_{step\text{-}by\text{-}step}$	50	51.20
Route $C_{one\text{-}pot}$	44	46.50

Further, minimal number of solvents used, no addition of protection groups and reagents providing a high AE received fewer penalty points and thus higher Green Motion™ 'reaction' scores.

Route $A_{subordinate}$ makes use of green catalyst TEMPO with a short reaction time of 9.50hours whereas route $B_{subordinate}$ does not make use of any catalyst and has a much longer reaction time of 30 hours. Further, route $A_{subordinate}$ and route $B_{subordinate}$ entailed a reaction selectivity step through the addition of benzyl (Bn) and carboxybenzyl (Cbz) protection group respectively to ASM compound, receiving penalty points. Additionally, both subordinate routes had fairly low overall reaction yields of 67%. Route $A_{subordinate}$ received additional penalty points for making use of BnCl that has toxic, corrosive and health hazard GHS pictograms as well as Et_2O that has flammable and harmful GHS pictograms. However, since route $A_{subordinate}$ had a much shorter reaction time and made use of a catalyst it received a better Green Motion™ 'reaction' score of 60 compared to route $B_{subordinate}$ of 55.

Moreover, the advantages of both route B procedures lie in the hydrogenation reactions (in the second step of the synthesis Scheme 4 and Scheme 5), that use low molecular weight (2.02g/mol) hydrogen gas as the reagent. This is considered an excellent reagent as it completely forms part of the bis-THF target compound and thus the reaction produces no waste, returning desired AE values and hence a higher Green Motion™ 'reaction' rating. Both route $B_{step\text{-}by\text{-}step}$ and route $B_{one\text{-}pot}$ synthetic procedures used three solvents (EtOAc, MTBE and c-hexane) all of which fall into one solvent classification namely petrochemical solvents, receiving fewer penalty points than multiple solvent classifications. The reason for route $B_{one\text{-}pot}$ procedure having a higher 'reaction' score is attributed to its large reduction in solvent quantity (420 kg less) compared to route $B_{step\text{-}by\text{-}step}$ procedure. Additionally, the efficient one-pot procedure and optimized reaction conditions (selection of correct reagent ratios, biocatalyst and photochemical wavelength) contributed to route $B_{one\text{-}pot}$ procedure's good 'reaction' score of 55. The disadvantage of both route $B_{step\text{-}by\text{-}step}$ and route $B_{one\text{-}pot}$ procedures lies in having the lowest overall reaction yields of 35% and 36% respectively, receiving penalty points. In contrast, route $A_{step\text{-}by\text{-}step}$ had the highest reaction yield of 81% with the longest reaction time of 54.50 hours, leading to the third highest 'reaction' score of 47.

Additionally, route $B_{step\text{-}by\text{-}step}$ procedure made use of PPL as a biocatalyst that resulted in a 10 hour and 7 hour reduced reaction time compared to route $A_{step\text{-}by\text{-}step}$ procedure using Pd/C catalyst and route $C_{step\text{-}by\text{-}step}$ procedure using DMF catalyst respectively. The PPL biocatalyst thus lowers the activation energy of the final reaction step in route $B_{step\text{-}by\text{-}step}$ procedure thus speeding up the rate of the chemical reaction to the bis-THF alcohol. Further, both route C procedures make use of excessively larger quantities of solvent materials and stoichiometric amounts of reagents, receiving higher penalty points and hence poor Green Motion™ 'reaction' scores of 25 and 35 for route $C_{step\text{-}by\text{-}step}$ and route $C_{one\text{-}pot}$ procedure respectively. To add, both route $C_{step\text{-}by\text{-}step}$ and route $C_{one\text{-}pot}$ synthetic procedures make use of six solvent types, five of which (EtOAc, PhMe, CPME, THF, DCM) are classified as petrochemical solvents and

one solvent (Me-THF) was classified as a renewable solvent, contributing to higher penalty points than route B with one type of solvent classification. Similarly, route $A_{step\text{-}by\text{-}step}$ comprises of six solvent types, four of which are categorized as petrochemical solvents (PhMe, THF, acetic acid and 2-propanol), one as a CMR solvent (MeOH) and one as a renewable solvent (PEG). It is however important to note that both route C procedures made use of potassium isocitrate as a starting material that has no associated GHS pictogram, making it a safe and non-hazardous starting material.

Overall it was found that reactions with reduced reaction times, high yields, catalysts/biocatalysts without protection and deprotection steps and minimal number of solvents materials and classifications automatically received better Green Motion™ 'reaction' scores.

Process category:

Considering the 'process' category, each bis-THF alcohol synthetic process was vastly different exhibiting unique merits and demerits. Route $A_{step\text{-}by\text{-}step}$ makes use of heating and cooling operations to 35℃ and 0℃ respectively, with reaction steps operating under pressure (at hydrogen pressure of 0.5MPa). Further, liquid- liquid extraction operations were used to separate organic layers with the impure product distilled under reduced pressure (0.132 – 0.12kPa and 72 - 197℃) in the final synthesis step 3a. Route $A_{step\text{-}by\text{-}step}$ thus returned an average Green Motion™ 'process' score of 40.

Both route $B_{step\text{-}by\text{-}step}$ and route $B_{one\text{-}pot}$ procedure operate at ambient temperatures and pressures thus receiving no penalty points for heating, cooling, or reaction steps under pressure. Route $B_{step\text{-}by\text{-}step}$ received penalty points for using flash column chromatography as well as rotary evaporation for solvent removal. It is important to note that there was no Green Motion™ option for chromatographic separation and thus other energy intensive method was selected (as described in Table 20). This however still returned favourable scores for route B suggesting that the penalty points received for this operation were too lenient. Owing to this and the merit of both route B procedures operating at ambient conditions a high Green Motion™ 'process' score of 85 is returned for both route B procedures.

In contrast, both route $C_{step\text{-}by\text{-}step}$ and route $C_{one\text{-}pot}$ procedure have heating and cooling and reaction steps that operate under pressure, receiving more penalty points than route A and B. Route $C_{step\text{-}by\text{-}step}$ procedure makes use of cooling to -5℃ (using water or glycol-water cooling) and heating up to 105℃ (using steam at 1 bar or oil bath). At the final synthesis step 5c atmospheric distillation at 80℃ was conducted. Further, route $C_{one\text{-}pot}$ procedure makes use of cooling to -8℃ (using water or glycol-water cooling) and heating up to 108℃ (using steam at 1 bar or oil bath). After synthesis step 2c (one-pot) atmospheric distillation was performed at 106℃ and after final step 3c a second atmospheric distillation was performed at 80℃. For both route $C_{step\text{-}by\text{-}step}$ and route $C_{one\text{-}pot}$ procedure, rotary evaporation was used to remove solvents as well as other energy intensive methods including crystallization and continuous extraction. Some of the reaction steps required mixtures to be heated under reduced pressure.

Owing to the large variety of energy intensive process operations, both route $C_{step\text{-}by\text{-}step}$ and route $C_{one\text{-}pot}$ procedures received low Green Motion™ 'process' scores of 30.

Regarding the 'process' category for subordinate routes, route $A_{subordinate}$ makes use of cooling operations to 0℃ using water baths, liquid- liquid extraction operation to separate the organic layers and fractional distillation at 80℃ to purify the intermediate compound at the end of synthesis step S1a (Scheme 1). The Green Motion™ 'process' score was thus returned at a value of 70. Route $B_{subordinate}$ comprises of heating to 25℃ using steam at 1 bar and cooling to 0℃ using a water bath. Additionally, the reaction was distilled under reduced pressure (105℃ and 0.2bar) and rotary evaporation was used to remove solvent at the final synthesis step S2b. The Green Motion™ 'process' score was thus returned at a value of 55.

On a side note, it is important to consider that the specific heat capacity of oil is lower than that of water $\left(C_{p_{oil}} = 1.67 - 2.50\ {}^{kJ}/_{kg℃}\ \text{and}\ C_{p_{water}} = 4.22\ {}^{kJ}/_{kg℃}\ (\text{Globe Core}, 2019)\right)$ thus less heat is required to increase the temperature of the oil bath. This suggests that oil is a more energy efficient and less environmentally impactful method of heating than water, but water is inherently safer than oil with fewer waste disposal issues being sent to water treatment plants.

Additionally, rotary evaporation[8] (commonly reported in routes B and C) is an environmentally friendlier solvent removal method similar to that of reduced pressure distillation (Nichols, 2019). It is energy efficient with a quick solvent removal design that can be easily scaled up. Labmate (2012), reported large scale rotary evaporators to reduce process cost whilst protecting the environment. The costs of buying and disposing of solvents has been on the increase thus recycling solvents using rotary evaporation illustrated a more sustainable solution.

Overall, both route B procedures had the highest Green Motion™ 'process' category scores at 85, being 55 points higher than lower performing route C processes. This was predominantly attributed to higher 'process' ratings associated with ambient temperatures and pressures, without additional energy intensive operations (liquid – liquid extraction, chromatography and so forth) and final work-up or distillation procedures.

Waste category:

For the 'waste' category the sE-Factor was selected as measure of waste generation. The sE-Factor of route $A_{subordinate}$ and route $A_{step\text{-}by\text{-}step}$ was 1.06 and 7.27 respectively. The waste impact values were low owing to small material quantities reported in both route $A_{subordinate}$ and route $A_{step\text{-}by\text{-}step}$, both resulting

[8] Rotary evaporation procedure requires a solution filled flask set in a water bath and rotated whilst being partially evacuated under reduced pressure at 0 ℃ and 70 ℃ (Nichols, 2019; Environmnetal Health and Safety, 2019). The reduced pressure allows for the solvent to boil at a lower temperature with the rotation motion speeding up the evaporation rate by increasing the liquid's surface area.

in high Green Motion™ 'waste' category scores of 87. Further, the sE-Factor for route $B_{subordinate}$, route $B_{step\text{-}by\text{-}step}$ and route $B_{one\text{-}pot}$ were 10.50, 10.49 and 13.64 respectively. The reason for the one-pot procedure having more waste is attributed to it requiring slightly larger quantities of ASM and reagent materials (~ 3 kg's) than the step-by-step procedure and since the sE-Factor does not account for solvent materials the one-pot waste is greater. The Green Motion™ 'waste' category scores for route $B_{subordinate}$, route $B_{step\text{-}by\text{-}step}$ and route $B_{one\text{-}pot}$ were returned values at 80, 80 and 67 respectively. The favourable scores of the route $B_{step\text{-}by\text{-}step}$ and the route $B_{one\text{-}pot}$ procedure were attributed to the photochemical reactions, which require a limited number and quantity of chemical reagents to perform successfully. This is directly linked to reduced waste generation and reaction cost.

Moreover, the sE-Factor of route $C_{step\text{-}by\text{-}step}$ and route $C_{one\text{-}pot}$ was 71 and 207 respectively, returning a waste score of 53 and 0 respectively. The high sE-Factor of route $C_{one\text{-}pot}$ was attributed to the use of higher material quantities. For instance, route $C_{one\text{-}pot}$ makes use of an additional reagent oxalyl chloride as well as an increase in DMF catalyst and DCM and CPME solvents quantities that resulted in more than two times greater waste generation than route $C_{step\text{-}by\text{-}step}$ procedure. Additionally, the one-pot procedure did not replace the entire route $C_{step\text{-}by\text{-}step}$ synthetic pathway but rather a part thereof (Scheme 7) and was not reported to perform under optimized reaction conditions. It is owing to the high sE-Factor of route $C_{one\text{-}pot}$ procedure that the maximum assigned Green Motion™ penalty points were reached thus returning a 'waste' score of 0.

Total score:

Considering the main synthetic pathways to bis-THF alcohol (excluding subordinate routes), route $B_{one\text{-}pot}$ procedure was found to be the overall best performing synthetic pathway with the highest total Green Motion™ score of 34. This was attributed to the synthetic pathway having a unique combination of green chemistry techniques including one-pot procedure, bio-catalysis, ambient reaction temperatures and pressures and renewable raw materials originating from woody bio-mass. Proceeding route $B_{one\text{-}pot}$ was route $B_{step\text{-}by\text{-}step}$ with a total score of 29. Thereafter route A, $C_{step\text{-}by\text{-}step}$ and $C_{one\text{-}pot}$ procedure followed with total scores of 24, 8 and 4 respectively. The excessively low values of route C were attributed to a variety of energy intensive process operations (atmospheric distillation, crystallization, continuous extraction, reactions under reduced pressure, heating and cooling), stoichiometric amounts of reagents used, large number and quantity of solvents used resulting in high waste generation. This suggests that route $B_{one\text{-}pot}$ procedure is the most sustainable and low impact synthetic pathway that takes human and environmental safety into high consideration, with route $C_{one\text{-}pot}$ being the most unsustainable and waste generating pathway to bis-THF alcohol.

Regarding the subordinate pathways, the total Green Motion™ scores for routes $A_{subordinate}$ and $B_{subordinate}$ were 35 and 31 respectively. This implies that route $A_{subordinate}$ was less environmentally impactful and more sustainable in ASM synthesis, scoring higher in all three concepts assessed.

5.5 Radial Polygon

There is no single green chemistry metric or tool that incorporates the multiple facets of process greenness and sustainability at the manufacturing stage of API's. For example, iGAL™ and Green Scorecard do not determine synthesis cost and which material category had the greatest mass contribution to waste generation. The SI metric does not account for the nature of the solvent materials and similarly the cE-Factor metric assigns all waste types, including solvent waste, the same impact weighting. The GSK solvent guide assesses the nature of the solvent and not the mass by using a traffic light inspired colour coding, based on multiple sustainability factors, and so forth. Thus, selecting a combination of green chemistry metrics, tools and guides provides a well-rounded comparative environmental impact assessment in synthesizing bis-THF alcohol.

Radial polygons combine all of the assessments selected and provide an overview as to which synthetic pathway performed best in terms of multivariable greenness performance. Figure 17 below illustrates a concise visual compilation of radial polygons and green performance criteria in assessing synthesis routes A, B and C.

Route $B_{one\text{-}pot}$ procedure showed the least distorted radial polygon ranking highest in cost, Green Motion™, AE, purity (*ee*%) and wastewater intensity (WWI) performance criteria. Following route $B_{one\text{-}pot}$ was route $B_{step\text{-}by\text{-}step}$ procedure performing equally in AE, purity (*ee*%) and WWI. The weakness of both route $B_{one\text{-}pot}$ and $B_{step\text{-}by\text{-}step}$ procedure lies in their low overall yields at 35% and 36% respectively. This is a reflection of the fact that route B procedure has a late stage kinetic resolution at the last stage of the synthesis which according to Sheldon (1996) is the worst case scenario as the 'golden rule' is to introduce the resolution step as early as possible in the synthesis. The late resolution step thus has a negative effect on all waste materials including solvents as illustrated in the polygon i.e., a high E-Factor and SI. Indeed, if an early resolution step was combined with a significant reduction in solvent use Route B would have an ideal radial polygon.

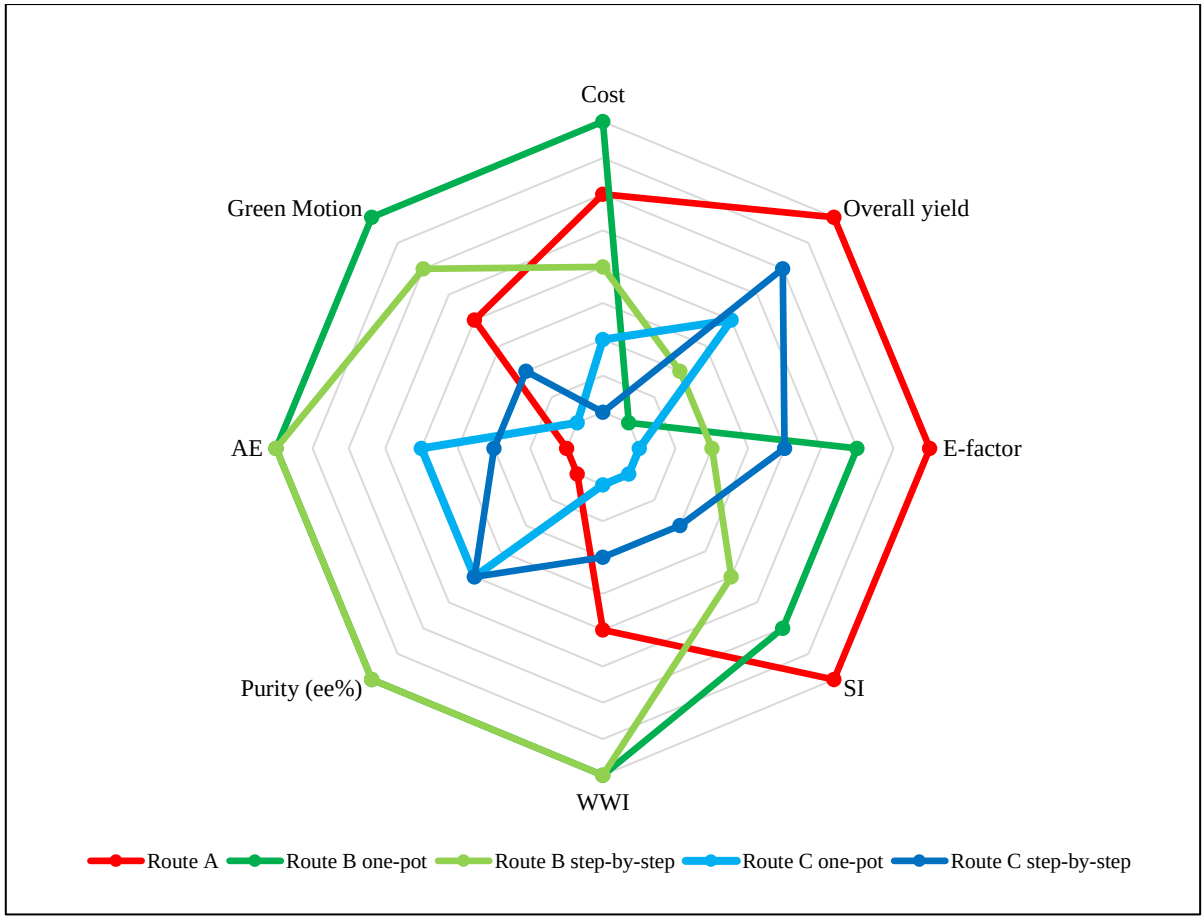


Figure 17: Radial octagon comparing multivariable greenness performance of synthesis routes to bis-THF alcohol

Route $A_{step\text{-}by\text{-}step}$ was the best performing synthetic pathway in overall yield (54%), SI and E-factor owing to the low material masses required in manufacturing the bis-THF alcohol. This raises an interesting point of discussion as overall yield is considered the universal mass metric and although important it disregards many other factors that may contribute towards a sustainable synthetic pathway. Conversely, both route C procedures are represented as the most distorted polygons, suggesting that they are the least green synthetic pathways to the bis-THF alcohol compared to both route $A_{step\text{-}by\text{-}step}$ and both route B procedures. Despite this, it is important to consider that route C has a very early resolution as the synthesis starts with optically pure materials thus having a good E-Factor, SI and overall yield.

As defined in the methodology, the outermost polygon is representative of the best performing synthesis route regarding process greenness. Hence a regular polygon of this size would portray an ideal green performance at 100%. Appendix J shows the data used in plotting the polygons along with each synthetic pathway area coverage percentage.

In summary, route $B_{one\text{-}pot}$ showed the greatest correlation to that of ideal with an 85% polygon area coverage. Following route $B_{one\text{-}pot}$ was route $B_{step\text{-}by\text{-}step}$ showing the second strongest greenness

performance correlation at 72.50%. Following route A, route $C_{step\text{-}by\text{-}step}$ and route $C_{one\text{-}pot}$ showed 67.50%, 47.50% and 37.50% area correlation respectively. It is imperative to note that the percentages are merely representative of the ranking system used in generating the radial polygons. The percentage value is a simplified representation of each radial polygon allowing for ease of comparison in terms of multivariable greenness performance.

5.6 Merits and Demerits of Synthesis Routes A, B and C

Table 22 and Table 23 summarizes the merits and demerits of entire routes A, B and C to bis -THF alcohol, respectively. The tables consider the ASM synthetic pathways as well as alternative process operations (step-by-step and one-pot procedures). The merits that are in alignment with one or more of the twelve principles of green chemistry are additionally indicated.

Table 22: Merits of routes A, B and C to bis-THF alcohol

	Route A	Route B	Route C
MERITS	Highest overall yield of 54%. Excellent mass efficiency with lowest E-Factor of 14.54 (principle 1). 9.09 times less wasteful than early stage API synthesis benchmark. Falls within the top 10% of green industrial pharmaceutical performance (principle 1). Green catalyst TEMPO (1 mol%) with a short reaction time of 9.50hours (principle 6 and 9). Environmentally benign D2PM organo-catalyst (principle 9). Green catalytic hydrogenation with Pd/C and stoichiometric reduction with low molecular weight $NaBH_4$ that produces little waste (principle 2, 8 and 9). Lowest solvent intensity of 48 kg/kg (principle 3). 66% of the solvents used were regarded as 'green', non-problematic solvents (principle 5 and 12). Starting material ethyl glyoxylate is inexpensive and could be directly used without pyrolysis making the synthesis highly practical, stereoselective and environmentally friendly with reduced energy demands (principle 6 and 8). Stereospecific cross adol reaction (asymmetric synthesis with max yield of 100% compare to kinetic resolution with max yield of 50%) (principle 8).	Shortest reaction time of 44 hours. Reaction operates at ambient temperatures and pressures (principle 4 and 6). No energy intensive operations (liquid – liquid extraction, chromatography or final work-up or distillation procedures) (principle 6). Rotary evaporation – quick, energy efficient solvent removal that reduces process cost. Photochemical reactions, which require a limited number and quantity of chemical reagents to perform successfully. Directly linked to reduced waste generation and reaction cost. (principle 3, 6 and 12) PPL biocatalyst which operates at low temperatures and pressures, allowing for multiple enzyme re-use and simple product recovery (principle 9). Optimized reaction conditions (principle 1, 6 and 8). Renewable raw materials originating from woody bio-mass (principle 7). Hydrogenation reactions that uses low molecular weight hydrogen (2.02g/mol) returning desired AE values (principle 2). Efficient one-pot procedure (principle 1, 3 and 8). Most economical. 80% of its total solvent mass were green solvents (EtOAc and MeOH), making it the safest and least impactful (principle 3 and 5). Highest polygon area coverage at 85% thus performing best across multiple greenness assessments. Good mass efficiency performance being 1.43 times less wasteful than early stage API synthesis benchmark. Falls within the top 30% of green industrial pharmaceutical performance (principle 1). Three solvents (MTBE, c-hexane and EtOAc) with favorable EHS characteristics and colour profiles used throughout the synthetic procedure allowing for ease of solvent recycling (principle 6). 80% of the total solvents used having a recommended solvent colour ranking (principle 5 and 12).	Early resolution step starting with optically pure potassium isocitrate that affords an optically pure bis-THF alcohol directly (principle 7 and 8). Potassium isocitrate has no associated GHS pictogram, making it a safe and non-hazardous starting material (principle 5). CPME and Me-THF bio-based ethers that have lower water solubility and less hazardous properties to classical ether solvents (principle 5 and 12).

Table 23: Demerits of routes A, B and C to bis-THF alcohol

	Route A	Route B	Route C
DEMERITS	Longest reaction time of 55 hours. Bn protection group (challenging to remove). Hazardous reagent BnCl. Hazardous solvent Et_2O with unfavorable colour (dark red) and GHS ratings. Multiple heating and cooling operations with reaction steps operating under pressure which limits industrial application. Liquid- liquid extraction and distillation were used to separate organic layers. Large variety of solvents used from all colour ranking categories. This may cause issue at later life-cycle stages as solvent mixtures require more expensive recycling, waste treatment or incinerated disposal. Moderate polygon area coverage at 67.50% thus performing moderately across multiple greenness assessments.	Kinetic resolution at final synthesis step. Lowest overall reaction yield at ~23%. Route $B_{\text{entire one-pot}}$, with E-factor values of 89.69 with synthesis status of 'good'. PPL is derived from animal origin (pig liver) which is unacceptable in the pharmaceutical industry. Cbz protection group.	Multiple heating and cooling operations with reaction steps operating under pressure which limits industrial application. Energy intensive operations including atmospheric distillation, crystallization and continuous extraction. No optimized reaction conditions. Excessively high solvent quantities used (> 500 kg) resulting in high waste generation. Stoichiometric amounts of reagents used. Large volumes of water during neutralization using ion exchange resin. Generates toxic and hazardous N-methyl aniline as a by-product. Least mass efficient with the highest E-Factor of 273.37. Below average mass efficiency performance being 2.14 times more wasteful than early stage API synthesis benchmark. Falls within the bottom 40% of green industrial pharmaceutical performance. Highest solvent intensity at 662 kg/kg. Only 5% of the solvents used were regarded as 'green' non-problematic solvents. Hazardous solvent DCM with unfavorable colour (red) and GHS ratings. Low polygon area coverage 47.50% thus performing least best across multiple greenness assessments.

Table 24, illustrates where each synthetic pathway performed better than its comparators in meeting the principles of green chemistry. Overall, it was found that route B performed best in most of the green chemistry principles and was the greenest synthetic pathway to the bis-THF alcohol compared to its comparators. Details pertaining to each synthetic pathways and the twelve principles of green chemistry are illustrated in Appendix K.

Table 24: Comparative checklist of 12 principles of chemistry for entire routes A, B and C

The 12 Principles of Green Chemistry	**Route A**	**Route B**	**Route C**
1. Waste prevention	Yes	Yes	No
2. Atom Economy	Yes	Yes	No
3. Less hazardous synthesis	No	Yes	No
4. Designing for safer products	N/A	N/A	N/A
5. Safer Solvents and Auxiliaries	No	Yes	No
6. Design for Energy Efficiency	No	Yes	No
7. Use of Renewable Feedstocks	No	Yes	Yes
8. Reduce Derivatives	No	Yes	Yes
9. Catalysis	Yes	Yes	Yes
10. Design for degradation	N/A	N/A	N/A
11. Real-time analysis for pollution prevention	N/A	N/A	N/A
12. Inherently safer chemistry for accident prevention	No	Yes	No

6 Conclusion

The 12 principles of green chemistry and definition of sustainability demonstrate that there are multiple factors that contribute towards process greenness. Thus, using any of the green mass metrics or tools in isolation does not drive sustainable environmental and business practices. Collectively using the metrics, tools and guides fully integrates the quantitative and qualitative information providing a well-rounded greenness assessment of the bis-THF alcohol synthetic pathways. Further, through careful selection of chemical materials and synthetic operations great contributions towards reduced waste generation and costs may be achieved. In combining the concluded findings from each green assessment, the most eco-efficient synthesis route was found.

Regarding the E-factor and waste generation, the following was further observed: The waste associated with synthesizing the ASM additively contribute to the total waste in synthesizing the bis-THF alcohol target molecule. Thus, in disregarding the synthetic pathway to the ASM a portion of waste in synthesizing the bis-THF alcohol would be neglected. Prior to considering the ASM synthetic pathway, the E-factor values for route $A_{step\text{-}by\text{-}step}$, $B_{step\text{-}by\text{-}step}$ and $B_{one\text{-}pot}$ procedures were 9.29 and 61.41 and 22.49 respectively. In adding the ASM E-factor the total E-factor values of route $A_{entire\ step\text{-}by\text{-}step}$, $B_{entire\ step\text{-}by\text{-}step}$ and $B_{entire\ one\text{-}pot}$ increased to 14.54, 126.77 and 89.69, respectively. In other words, in disregarding the synthetic pathway to the ASM 57%, 52% and 75% of the waste associated with route $A_{step\text{-}by\text{-}step}$, $B_{step\text{-}by\text{-}step}$ and $B_{one\text{-}pot}$ procedures would have been neglected. It was thus concluded that the definition of ASM was imperative in finding a common starting point for each synthetic pathway and determining the true E-factor values for entire synthetic pathway to the bis-THF alcohol. Route C did not have an ASM but had two procedures with E-factors of 124.84 and 273.37 for route $C_{step\text{-}by\text{-}step}$ and $C_{one\ \text{-}pot}$ respectively.

Comparing the best performance of the synthetic routes demonstrated that route $B_{entire\ one\text{-}pot}$ procedure had a 77% cE-factor reduction and 77% cost reduction when compared to route $C_{step\text{-}by\text{-}step}$ procedure. Further, route $B_{one\text{-}pot}$ procedure had a 74% increase in cE-Factor and a 43% cost reduction when compared to route $A_{step\text{-}by\text{-}step}$ procedure. Route $A_{entire\ step\text{-}by\text{-}step}$ was thus found to be the most mass efficient and route $B_{entire\ one\text{-}pot}$ the most cost effective synthetic pathways to the bis-THF alcohol.

From the iGAL^{TM} methodology, route $A_{entire\ step\text{-}by\text{-}step}$ was found to have 'excellent' mass-efficiency with a low E-factor of 14.54 followed by route $B_{one\text{-}pot}$ with a 'good' mass - efficiency and moderate E-factor of 89.69 and lastly route $C_{step\text{-}by\text{-}step}$ with an 'average' mass - efficiency and high E-factor of 124.84. In comparing synthetic pathways, the greatest waste reduction was found from route $C_{step\text{-}by\text{-}step}$ to route $A_{entire\ step\text{-}by\text{-}step}$ at 88%, mainly attributed to mass of water and solvent used, followed by the waste reduction between route $B_{one\text{-}pot}$ to route $A_{entire\ step\text{-}by\text{-}step}$ at 84%, only attributed to mass of solvent use.

Considering the impacts of solvents, solvent materials were found to be the largest waste contributors with the greatest environmental concern in all three synthetic pathways. More specifically of the total

material mass, solvent materials contributed 45% to route $A_{step\text{-}by\text{-}step}$, 84% to route $B_{one\text{-}pot}$, 96% to route $B_{step\text{-}by\text{-}step}$, 41% to route $C_{one\text{-}pot}$ and 46% to route $C_{step\text{-}by\text{-}step}$ procedure. Thus, solvent utilization offers the greatest potential in reducing the environmental impacts (E-factor) of bis-THF alcohol synthesis both qualitatively and quantitatively.

More specifically, route $A_{entire\ step\text{-}by\text{-}step}$ has the smallest solvent mass intensity (48 kg/kg product) and route C comprises of ≥95% hazardous and problematic solvents with >500 kg total solvent mass required per 1 kg bis-THF alcohol, making it the most costly and wasteful synthetic pathway with health and safety issues. Conversely, 80% of the total solvent mass used in route $B_{entire\ one\text{-}pot}$ procedure were green solvents (EtOAc and MeOH), making it the least impactful and safest synthetic pathway from a qualitative solvent perspective. Significant cost savings were associated with these solvents as route $B_{entire\ one\text{-}pot}$ procedure showed the cheapest solvent cost, in manufacturing of 1 kg bis-THF alcohol saving up to 51% solvent costs compared to route A and C procedures. This suggests that route $B_{entire\ one\text{-}pot}$ procedure has the smallest environmental footprint.

From the Green Motion™ tool, it was found that reactions with reduced reaction times, high yields, catalysts/biocatalysts with minimal amounts of solvents materials operating at ambient conditions that were less waste generating (low E-factors) returned more favourable Green Motion™ scores. Route $B_{one\text{-}pot}$ had the highest total Green Motion™ score of 34 suggesting that it is the greenest and most sustainable synthetic pathway to the bis-THF alcohol. This was attributed to the synthetic pathway having a unique combination of green chemistry techniques including one-pot procedure, bio-catalysis, ambient reaction temperatures and pressures, reasonable amounts of green solvents and renewable raw materials originating from woody bio-mass. Conversely, route $C_{one\text{-}pot}$ procedure represented the lowest total Green Motion™ score of 4 suggesting that it is the least sustainable and most waste generating pathway to the bis-THF alcohol. The excessively low values of route C were attributed to a variety of energy intensive process operations (atmospheric distillation, crystallization, continuous extraction, reactions under reduced pressure, heating, and cooling), stoichiometric amounts of reagents and large amounts of solvents, resulting in high waste generation.

From the collective radial polygon comparator, it was found that route $B_{one\text{-}pot}$ procedure covered the highest polygon area at 85% thus performing best across multiple greenness assessments and the greenest synthetic pathway. The radial polygon rates the synthetic pathways from most green to least green, across multiple greenness assessments, as: route $B_{one\text{-}pot}$, route $B_{step\text{-}by\text{-}step}$, route $A_{step\text{-}by\text{-}step}$, route $C_{step\text{-}by\text{-}step}$ and route $C_{one\text{-}pot}$ procedure.

In conclusion, route $B_{one\text{-}pot}$ procedure was found to be the most efficient, scalable, inexpensive, environmentally friendly, and least health hazardous pathway in assembling the bis-THF alcohol. It adhered to several principles of green chemistry through the use of renewable, CO_2 neutral wood based materials (xylochemistry), acceptable and non-chlorinated solvents (for waste minimization and safety),

photochemical reactions and bio-catalytic reactions at ambient operating conditions. Route $B_{one\text{-}pot}$ procedure quantitatively supports the triple win of green chemistry by demonstrating:

a. Cost effectiveness through a 77% and 43% cost reduction compared to routes $C_{step\text{-}by\text{-}step}$ and route $A_{entire\ step\text{-}by\text{-}step}$ respectively,
b. Environmental friendliness with a good E-factor value of 90 which was below the average benchmarked API GCIPR E-factor value of ~130
c. Safety as 80% of the total solvents used were highly acceptable with green colour ranking implying few environmental, health and safety issues. Further, the use of less hazardous reagents and ambient temperature and pressure operating conditions.

Overall, route $B_{entire\ one\text{-}pot}$ procedure was found to be the most sustainable pathway scoring the best amongst all assessment criteria (radial polygon) and the most eco-efficient demonstrating significant cost saving and reduced environmental waste burden compared to the other synthetic pathways.

7 Recommendations

7.1 Recommendations for the most eco-efficient pathway route B:

Route B exhibits the greatest potential in being the greenest synthesis pathway to the bis-THF alcohol. Despite this, the synthesis route has a large E-factor almost entirely attributed to the large amounts of solvent (c-hexane/ EtOAc) used in the chromatographic separation techniques. Significant environmental and economic demerits lie in the final enzymatic resolution step of the racemic bis-THF alcohol. The demerits include:

- Conducting the enzymatic resolution step with propionic anhydride resulting in an alcohol and ester products of similar physical properties. Separation of these products requires flash chromatography, which is solvent intensive, expensive and not suited for large scale manufacturing.
- The use of porcine pancreas lipase (PPL) enzyme, an animal derived enzyme, in the synthesis of API's is prohibited by regulatory agencies such as the FDA.
- The process has a late stage resolution which means more waste from solvents and reagents and higher cost than with an earlier stage resolution (Sheldon, 1996).

In attempt to solve the above-mentioned demerits, the following recommendations are suggested:

- Conducting the enzymatic resolution step with succinic anhydride instead of propionic anhydride to yield alcohol and acid half ester products with non-similar physical properties. The bis-THF alcohol might however need to be salted out with saturated NaCl solution.
- The use of *Pseudomonas cepacia* lipase (PcL), a plant derived enzyme, instead of PPL. The PcL enzyme was reported as an appropriate, optimum enzyme by Sevenich *et al.*, (2017). The PcL is commercially available and has been used in combination with succinic anhydride in alcohol resolution (Melais, Boukachabia, Aribi-Zouioueche and Riant, 2015).

For solvent analysis, it is recommended that one solvent guide be used to ensure consistency in interpretation of relative solvent greenness.

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